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## **Energetic Consequences of Human Impacts for Bioindicator Atlantic Ghost Crab (*Ocypode Quadrata*)**

Mustafa Remzi Gül

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ENERGETIC CONSEQUENCES OF HUMAN IMPACTS FOR  
BIOINDICATOR ATLANTIC GHOST CRAB (*OCYPODE QUADRATA*)

by

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## DEDICATION

This dissertation is dedicated to my family, my father Eyüp, my mother Ayşe, my sisters Fatma and Zeynep and my wife Esra for their love and support.

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I would like to thank my family who have always supported during this long journey. My parents Eyüp Gül and Ayşe Gül, my sisters Fatma and Zeynep, and my wife Esra: your support and encouragement made this work possible. I would like to thank members of Griffen Lab, especially Zac Cannizzo, Ben Belgrad, Eilea Knotts. I would also like to thank Ahmet Postaağası, Paul Kenny and Matt Kimball. I would also like to thank Baruch Institute, Coastal Carolina University, Tom Yawkey Wildlife Center, and DeBordieu Colony management for permission to access their facilities.

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## ABSTRACT

Human activities have caused notable changes in ecosystems globally over the past century, which cause substantial ecological issues. The degree of these ecological issues is often investigated using the responses of bioindicator species. Bioindicator species often reduce their population densities and individual sizes as a response to the human disturbance. Observing these variations in the demographics is often an effective way to measure the strength of the ecological changes. However, they do not provide any mechanistic reason (s) for the shifts in the demographics, which is important for making predictions about responses of the species to the future conditions. Therefore, this dissertation employs an energetic approach to investigate the human disturbances on a bioindicator species, *Ocypode quadrata*. We show that *O. quadrata* populations alter their demographics as a response to the human disturbance, suggesting a reliable bioindicator for sandy shores. We also show that organisms alter their behavior based on the strength of the human disturbance, and these behavior changes are the energetic consequences of the human disturbance. We further show that human disturbance has a selective pressure on the morphology of *O. quadrata*. By combining field and laboratory surveys, we also show that habitat quality varies with relation to the strength of human disturbance and these habitats with lower quality influence the diet, physiology and reproductive potential of *O. quadrata*. Ultimately, this dissertation highlights the influences of human disturbance on ecology and physiology of a bioindicator.

## TABLE OF CONTENTS

Dedication .....	iii
Acknowledgements .....	iv
Abstract .....	v
List of Tables .....	viii
List of Figures .....	ix
Chapter 1: Introduction .....	1
Chapter 2: A reliable bioindicator of anthropogenic impact on the Coast of South Carolina.....	8
Chapter 3: Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores.....	25
Chapter 4: Combined impacts of natural and anthropogenic disturbances on the bioindicator <i>Ocypode quadrata</i> (Fabricius, 1787).....	65
Chapter 5: Changes in claw morphology of a bioindicator species across habitats that differ in human disturbance .....	90
Chapter 6: Burrowing behavior and burrowing energetics of a bioindicator under human disturbance .....	119
Chapter 7: Human impacts change diet, energy storage, and reproduction in bioindicator species .....	157
Chapter 8: General conclusion.....	192
Appendix A: Methodological supplement for CHAPTER 6 .....	200
Appendix B: Supplemental tables for CHAPTER 6.....	202
Appendix C: Supplemental tables and figures for CHAPTER 7 .....	205
Appendix D: Permission for republication of CHAPTER 2.....	209

Appendix E: Permission for republication of CHAPTER 3 .....	210
Appendix F: Permission for republication of CHAPTER 4 .....	211



## LIST OF TABLES

Table 2.1 Statistical outputs for results presented in burrow density and size study.....	17
Table 3.1 Statistical outputs for results of burrow density .....	47
Table 3.2 Statistical outputs for results of burrow dimensions.....	48
Table 5.1 Description of study sites and UI.....	104
Table 5.2 Statistical output for results of claw morphology .....	105
Table 6.1 Description of study sites and UI index.....	140
Table 6.2 Statistical output for results of burrow longevity .....	141
Table 7.1 Statistical output for habitat quality .....	177
Table B.1 Levels and score for UI index .....	203
Table B.2 Study sites and geo-morphological characteristics .....	204
Table C.1 Study sites and sample size of individuals .....	205
Table C.2 Results of post-hoc pairwise comparison .....	206
Table C.3 Results of PERMANOVA for prey selection .....	207

## LIST OF FIGURES

Figure 2.1 Location of the study sites .....	18
Figure 2.2 Burrow density and size under human disturbance .....	19
Figure 2.3 Changes in sex-ratio of the crabs under human disturbance .....	20
Figure 3.1 Study sites.....	49
Figure 3.2 Changes in the burrow density and size under human disturbance .....	50
Figure 3.3 Variation in burrow diameter across different levels of human disturbance....	51
Figure 3.4 Variation in burrow density across different levels of human disturbance .....	52
Figure 3.5 Variation in burrow shapes across different levels of human disturbance .....	53
Figure 3.6 Variations in burrow metrics among impact types.....	54
Figure 3.7 Environmental properties .....	55
Figure 4.1 Map of the study sites.....	80
Figure 4.2 Variation in burrow density and size pre- and post-hurricane .....	81
Figure 4.3 Variation in the burrow density pre- and post-hurricane.....	82
Figure 4.4 Size frequency variation of burrows pre- and post-hurricane .....	83
Figure 5.1 Measured claw dimensions for ghost crabs.....	106
Figure 5.2 Variation in residual major claw dimensions of ghost crabs.....	107
Figure 5.3 Variation in residual minor claw dimensions of ghost crabs .....	108
Figure 5.4 Variation in residual claw closing force of ghost crabs .....	109
Figure 6.1 Relationship between burrow fidelity and burrow size.....	142
Figure 6.2 Relationship between burrow longevity and burrow size .....	143

Figure 6.3 Glycogen level based on burrow size and between experimental groups .....	144
Figure 6.4 Variation in residual calculated annual burrowing energy demand .....	145
Figure 7.1 Variation in the abundance of diet items for ghost crabs .....	178
Figure 7.2 Contribution of prey items to the diet.....	179
Figure 7.3 Variation in the % frequency of diet items and in the gut fullness .....	180
Figure 7.4 Variation in $\delta^{15}\text{N}$ and gut-width:carapace-width ratios.....	181
Figure 7.5 HSI and GSI values of crabs among various disturbance levels .....	182
Figure 7.6 Variation in aggression level, HSI and GSI values from feeding survey.....	183
Figure C.1 NMDS plot for prey selection of ghost crabs across disturbance levels .....	208

# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Human activities, as a major force, have caused notable changes in ecosystems globally over the past century (Vitousek et al. 1997, Halpern et al. 2008), which vary from tourism to pollution and urbanization (Davenport & Davenport 2006). These activities directly influence populations by altering diversity and abundance (Lotze et al. 2006, Schooler et al. 2019), species interactions (Maxwell et al. 2013), land-use dynamics (Ojima et al. 1994), polluting ecosystems (Schwarzenbach et al. 2010) and degrading the habitats (Liu et al. 2007).

The degree of these human influences on ecosystems is widely examined by using bioindicator species (Carignan & Villard 2002, Spellerberg 2005, Heink & Kowarik 2010, Cortes et al. 2013, Siddig et al. 2016). Presence/absence, abundance, or age/size structure of bioindicator species are often observed as the responses of to the human disturbances, because these observations allow use of methods that require reduced time, cost and effort (Carignan & Villard 2002, Spellerberg 2005, Heink& Kowarik 2010, Siddig et al. 2016). Reliable knowledge related to the ecological changes can be obtained by observing these variations in population demographics of bioindicator species under human stress (Carignan & Villard 2002, Spellerberg 2005, Cortes et al. 2013), however, they do not provide any mechanistic understanding of the link between human

disturbances and these demographic changes. Thus, the overall mechanistic reason(s) of the changes in population demographics are not quite understood, and achieving this understanding is important for forecasting the future conditions of the populations when the human disturbance is expected to be stronger as the size of human population increases (Vitousek et al. 1997).

The objective of this dissertation is to expand our mechanistic understanding of the responses of bioindicator species to the human disturbances. I accomplish this by focusing on variation in behavioral, morphological and physiological responses to the human disturbances. Here I used The Atlantic ghost crab, *Ocypode quadrata*. *O. quadrata* is an important member of sandy shores by providing a trophic link from smaller invertebrates and wrack material to the higher predators (Wolcott 1978, Fisher & Tevesz 1979). In addition, *O. quadrata* is a widely accepted bioindicator species (Wolcott & Wolcott 1984, Neves & Bemvenuti 2006, Hobbs et al. 2008). As a general trend, like the other bioindicator species, *O. quadarata* respond human disturbances by lowering their population densities and individual size, and the degree of these changes in population demographics are correlated the strength of the human disturbance (Schlacher et al. 2016).

This dissertation expands our understanding of human influences on the ecology, population demographics, morphology and behavior of bioindicator species by focusing on the following:

CHAPTER 2 examines variations in the population demographics of *O. quadrata* with relation to the human disturbance and geo-morphological variables including sand

compaction and sand grain size. Specifically, this chapter explores how the level of responses vary at the sites that differ in the strength of the human disturbance and geomorphological characteristics. Variation in the individual sizes, population densities and sex-ratios are investigated through field observations.

CHAPTER 3 investigates the population responses to the various levels of human disturbances in terms of distribution, population demographics and behaviors. It does so by examining the influences of human disturbances on the responses of the crabs in terms of their distribution pattern within a beach, burrowing behavior, and population demographics, and the interactions of those responses at sites that differ in the extend of human disturbance.

CHAPTER 4 examines another aspect of the human disturbances, which may limit the ability of the populations to persist when they face extreme natural disturbances. This chapter investigates the potential consequences of the combined anthropogenic and natural disturbances on the population densities, individual sizes and population distribution through pre- and post-hurricane surveys. Specifically, this chapter examines whether human disturbances make the populations less resilient against natural disturbances.

CHAPTER 5 assesses variations in the morphological characteristics of *O. quadrata* as a result of the human disturbances. This chapter examines how claw morphology varies between sites that differ in the strength of the human disturbance and between sexes. In particular, it investigates the potential selective role of human disturbances on the morphology of individuals from different sexes.

CHAPTER 6 investigates variation in behavior and the potential energetic consequences. By combining field observations and experiments with laboratory work, this chapter assesses whether *O. quadrata* alter their burrowing longevity and fidelity and the energetics of burrowing across levels of human disturbance.

CHAPTER 7 examines whether human disturbance alters the habitat quality on the basis of food availability and type and whether these changes have a physiological and reproductive consequences for *O. quadrata*. It does so by conducting a set of field surveys, laboratory experiments, and physiological analyses that explore the physiological and reproductive consequences of diet strategy for *O. quadrata* across two consecutive years. This chapter specifically examines whether the variations in demographics investigated in CHAPTER 2 and CHAPTER 3 are the results of diet changes induced by human disturbance.

CHAPTER 8 stands as a general conclusion to the dissertation.

## **1.2 Literature Cited**

Carignan, V., & Villard, M.A. (2002). Selecting indicator species to monitor ecological integrity: a review. *Environmental Monitoring and Assessment*, 78(1), 45- 61. PMID: 12197640

Cortes, R.M.V., Hughes, S.J., Pereira, V.R., & Varandas, S.D.P. (2013). Tools for bioindicator assessment in rivers: the importance of spatial scale, land use patterns and biotic integration. *Ecological Indicators*, 34, 460- 477.

<https://doi.org/10.1016/j.ecolind.2013.06.004>

Davenport, J., & Davenport, J. L. (2006). The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine, Coastal and Shelf Science*, 67(1-2), 280- 292. <https://doi.org/10.1016/j.ecss.2005.11.026>

Fisher, J. & Tevesz, M. (1979). Within-habitat spatial patterns of *Ocypode quadrata* (Fabricius) (Decapoda Brachyura). *Crustaceana*, 5, 31- 36.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., ....Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, 319, 948- 952.

Liu, J., Dietz, T., Carpenter, S.R., Alberti, M., Folke, C., Moran, E.,....Taylor, W.W. (2007). Complexity of Coupled Human and Natural Systems. *Science*, 317, 1513. DOI: 10.1126/science.1144004

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C.,....Jackson, J.B.C. (2006). Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. *Science*, 312, 1806-1809.

Maxwell, S.M., Hazen, E.L., Bograd, S.J., Halper, B.S., Breed, G.A., Nickel, B.....Costa, D.P. (2013). Cumulative human impacts on marine predators. *Nature Communications*, 4, 1-9. doi:10.1038/ncommsS3688

Ojima DS, Galvin KA, Turner BL II. (1994). The global impact of land-use change. *BioScience*. 44(5): 300–304. <https://doi.org/10.2307/1312379>

Schlacher, T. A., Lucrezi, S., Connolly, R. M., Peterson, C. H., Gilby, B. L., Maslo, B.,..... Schoeman, D. S. (2016). Human threats to sandy beaches: A meta-analysis of



ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal and Shelf Science*, 169, 56- 73. <https://doi.org/10.1016/j.ecss.2015.11.025>

Siddig, A.A.H., Ellison, A.M., Ochs, A., Villar-Leeman, C., & Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in ecological indicators. *Ecological Indicators*, 60, 223-230. doi:10.1016/j.ecolind.2015.06.036

Schooler, N.K., Dugan, J.E., & Hubbard, D.M. (2019). No lines in the sand: Impacts of intense mechanized maintenance regimes on sandy beach ecosystems span the intertidal zone on urban coasts. *Ecological Indicators*, 106, 105457. <https://doi.org/10.1016/j.ecolind.2019.105457>

Schwarzenbach, R.P., Egli, T., Hofstetter, T.B., von Gunten, U., & Wehrli, B. (2010). Global Water Pollution and Human Health. *Annual Review of Environment and Resources*, 35, 109- 136. 10.1146/annurev-environ-100809-125342

Spellerberg, I. (2005). *Monitoring ecological change* (2nd ed.). Cambridge, UK: Cambridge University Press.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of the Earth's ecosystems. *Science*, 277, 494–499.

Wolcott, T.G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fab.) on an ocean beach: scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31, 67–82.

Wolcott, T.G., & Wolcott, D.L. (1984). Impact of off-road vehicles on macroinvertebrates on a Mid-Atlantic beach. *Biological Conservation*, 29(3), 217- 240.  
[https://doi.org/10.1016/0006-3207\(84\)90100-9](https://doi.org/10.1016/0006-3207(84)90100-9)

CHAPTER 2

A RELIABLE BIOINDICATOR OF ANTHROPOGENIC IMPACT ON  
THE COAST OF SOUTH CAROLINA<sup>1</sup>

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<sup>1</sup>Gül MR. & Griffen BD. 2018. A Reliable Bioindicator of Anthropogenic Impact on the Coast of South Carolina. *Southeastern Naturalist*.17(2): 357-364.  
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## **2.1. Abstract**

Sandy beaches are frequently visited for recreational purposes. Although such recreational activities are economically beneficial, they cause disturbances to these habitats. Every ecosystem has unique properties; thus, ecosystem-specific species are often used as bioindicators of human disturbances. Here, we pioneer the use of an indirect burrow-counting technique on 20 sandy South Carolina beaches that experience different levels of human disturbance. Our results show that *Ocypode quadrata* (Atlantic Ghost crab) is a reliable bioindicator of human disturbance on sandy beaches in South Carolina. The burrow density and width declined significantly as human disturbance increased. We also report that females outnumber males in highly disturbed sites. We conclude that the indirect burrow-counting technique employed in this study could minimize the cost and the effort of determining the human disturbances on coastal regions.

## **2.2 Introduction**

Certain species have been defined as bioindicators because they are sensitive to ecological changes (Siddig et al. 2016). Collecting all possible data related to a habitat is not realistic (Carignan & Villard 2002, Pérez-García et al. 2016); thus, scientists and managers have increasingly used bioindicators to examine a variety of natural and human impacts across spatial and ecological scales (Carignan & Villard 2002, Cortes et al. 2013, Siddig et al. 2016). Indicator species provide useful but limited information about a viable species or a species assemblage at local and regional scales (Cortes et al. 2013), which is particularly helpful in the development of conservation and management plans (Solomon et al. 2003). Selecting an appropriate bioindicator species is especially critical in coastal systems

where evaluating human alterations in marine ecosystems is difficult (Vitousek et al. 1997). Thus, selection of an appropriate bioindicator species to determine the human impacts on long-stressed sandy beaches (Davenport & Davenport 2006, McLachlan et al. 2013) is important to assess the level and effects of human disturbance and determine if ecosystem services are being maintained (Barbier et al. 2011) in coastal regions.

Invertebrates are often chosen as bioindicators because they are more sensitive to human stresses than vertebrate taxa in terrestrial and aquatic systems (Carginan & Vilard 2002). For this reason, many invertebrate taxa have been used as bioindicators in a number of sandy-beach regions including *Donax* spp. (clams; Defeo & de Alava 1995, Schlacher et al. 2008, Sheppard et al. 2009), *Phaleria* spp. (coleoptera; González et al. 2014), *Emerita* spp. (mole crabs; Cardoso et al. 2016), and *Ocypode* spp. (ghost crabs; Schlacher et al. 2016a and citations). Ghost crabs, in particular, are used globally as bioindicators for sandy beaches (Schlacher et al. 2016a) due to their sensitivity to both human (Neves & Bemvenuti 2006) and natural impacts (Hobbs et al. 2008). Here, we examine the usefulness of *Ocypode quadrata* (Fabricius) (Atlantic Ghost Crab) as a bioindicator of human stress on South Carolina sandy beaches.

The Atlantic Ghost Crab is the only ghost crab species found on the sandy beaches of the western Atlantic (Milne & Milne 1946), with a range extending from Rhode Island to Brazil (Williams 1984). The mono-specific nature of ghost crabs in the western Atlantic increases their utility as a bioindicator by eliminating any possibility of species or burrow misidentification (Pombo & Turra 2013). Atlantic

Ghost Crabs also play an important ecological role in food webs on sandy beaches by consuming small invertebrates (Wolcott 1978), thus acting as an energetic link to higher trophic levels (Fisher & Tevesz 1979). Ghost crabs are often studied by examining their burrows on sandy beaches, because burrow width and abundance are well-established proxies for crab-population size and structure (Schlacher et al. 2016b). Examining the Atlantic Ghost Crab burrows provides a low-cost and efficient sampling design (Pombo & Turra 2013).

### **2.3 Study-site Description**

We selected our study sites (Fig. 2.1) based on differences in the intensity of human visitors, whether or not the beach experienced mechanical cleaning, and presence or absence of vehicles. We considered the sites that are accessible by only boat (North Island 1 and 2, South Island 1 and 2, and Debordieu Island) as pristine because we observed no pedestrians. In order to determine the intensity of human disturbance on the beaches, we used binoculars to count the number of pedestrians on each site over a 2-h period from 0800 to 1000 over ~1 linear kilometer of beach. We considered the sites to be highly impacted if the number of pedestrians was greater than 50 per hour. The average number of pedestrians was 39.3(SD =  $\pm 17.5$ ) for moderately impacted and  $60.2 \pm 32.08$  for highly impacted sites.

Although recreational off-road vehicles are not allowed on South Carolinian beaches, some highly visited sites, such as Myrtle Beach, are mechanically cleaned. At some sites, municipality agents use vehicles to empty garbage bins and collect the litter on dunes. Moreover, security agents and life-guards often use four-wheel-drive vehicles on the beaches. We also observed whether the sites were mechanically

cleaned and whether other vehicles were present during night hours. Some other sites, such as Surfside beach, are highly visited during peak tourism months and are mostly the destinations for extended vacations due to local accommodations and entertainment. In contrast, sites like Pawley's Island are mostly visited by locals and thus tend to experience little anthropogenic impact. Finally, we added some pristine sites to our study, such as South Island. These sites can only be accessed by boat and experience no anthropogenic impact.

The North Myrtle Beach 1 and 2, Myrtle Beach 1 and 2 and Garden City Beach sites experienced mechanical cleaning and high disturbance by pedestrians; we considered them highly disturbed by vehicles and people. We classified the Surfside Beach 1 and 2, Sullivan's Island, Isle of Palm 2, and Burkes Beach sites as highly disturbed sites by only people because they were not mechanically cleaned, and we observed no vehicles at those sites. We considered Pawley's Island 1 and 2, Folly Beach, Isle of Palm 2, and Edisto Beach to be moderately impacted sites due to the fact that they experienced only human impacts (less than 50 pedestrians per hour).

## **2.4 Methods**

To determine whether Atlantic Ghost Crab is a useful bioindicator species for South Carolina sandy beaches, we deployed 3 replicate rectangular transects (10 m x 5 m) parallel to the shore, randomly located within 2.5 m above and below the back-shore (i.e., dune) vegetation at each of the 20 sites. Transects were spaced ~200 m apart. We counted and used Vernier calipers to measure to the nearest 0.1 mm the width of the opening of all burrows within the transect. We sampled transects at each site just after sunrise to both diminish the impact of humans on the

burrows and to allow individual crabs to return to their burrows following their nocturnal activities.

In addition to anthropogenic impacts, morphological characteristics of sandy beaches such as sand compaction (Pombo and Turra 2013) and grain size (Defeo & McLachlan 2011) may also influence the density of Atlantic Ghost Crab burrows and the burrow width. We determined grain size at each site by sieving, which is a widely used method for sand-dominated material (Cheetham et al. 2008, Folk 1980). Specifically, we collected three 500-g sand samples from each site, which we then sieved through a variety of mesh sizes (2 mm, 1 mm, 0.5 mm, 0.42 mm, 0.25 mm, 0.177 mm, 0.125 mm and 0.074 mm). We measured the sand compaction with a pocket penetrometer (AMS, E-280, Zoro, Inc., Buffalo Grove, IL) 5 times at each site. Sand resistance was extremely low; thus, we used an adaptor foot on the penetrometer to increase the surface area of the measurement by 16-fold. At the end of each measurement, we divided our result by 16 to obtain the true compaction. To determine if the sex ratio of Atlantic Ghost Crabs differed between sites, we caught all opportunistically encountered individuals using a dip-net around the strand line over a distance of ~1 km for 2 h during a time of day when crabs are often out of their burrows (between 0000 and 0200).

To examine which factors impacted burrow density and diameter, we used separate generalized linear mixed models (GLMM) with poisson and gaussian distributions, respectively, with impact level, sand compaction, grain size, and latitude as predictor variables for both models. We also included sampling day to control for seasonal and daily (i.e., weather) changes and transects nested within sites to control



for the site differences as random effects in each model. We employed Akaike's information criterion (AICc) values to determine the best model. When there was more than 1 model with  $\Delta AICc < 2$ , these models were averaged (Burnham & Anderson 2002). We employed a chi-square test to examine the difference in sex ratio of observed Atlantic Ghost Crabs between sites with various impact levels. We conducted all statistical analyses in R, version 3.3.2 (R Core Team 2016).

## 2.5 Results

We found that human impacts had a significant influence on both burrow density and diameter of Atlantic Ghost Crabs on South Carolinian sandy beaches. Both the density (min–max = 0.3–1.146/m<sup>2</sup>) and opening diameter (min–max = 11.0–76.0mm) of the Atlantic Ghost Crab burrows declined significantly with increased human impact level (Table 2.1, Fig. 2.2). Latitudinal variation also significantly impacted the burrow diameter but not the density (Table 2.1). The burrow diameter significantly increased with the latitude. Sand compaction in the study sites varied between 0.071 kg/cm<sup>2</sup> (South Island 1) and 0.288 kg/cm<sup>2</sup> (Myrtle Beach 2) and the average sand-grain size at the sites was between 0.22 mm (Burkes Beach) and 0.38 mm (Pawley's Island 2). The sex ratio (males/females) varied from 0.88 at sites highly impacted only by people to 1.64 in pristine sites (chi-squared test:  $\chi^2 = 659.1, 8, P < 0.0001$ ). However, sites appeared to group into 2 distinct categories in terms of sex ratio; pristine and moderately impacted sites showed similar ratios (chi-squared test:  $\chi^2 = 0.10, P = 0.75$ ) and sites highly impacted by people only and highly impacted by both people and vehicles showed similar ratios (chi-squared test:  $\chi^2 = 0.079, P = 0.99$ ; Fig. 2.3).

## 2.6 Discussion

Here, we provided the first record of Atlantic Ghost Crab as a useful bioindicator species for human impacts on South Carolina sandy beaches. Atlantic Ghost Crabs provide useful information for human impacts by displaying a dramatic decline in burrow density and diameter in disturbed beaches. Similar decreases have been reported for Atlantic Ghost Crab on the coast of North Carolina at sites affected by off-road vehicles (Hobbs et al. 2008, Wolcott & Wolcott 1984), as well as in Texas (Maccarone & Mathews, 2007) and Brazil (Neves & Bemvenuti 2006). Our results are also consistent with the results of the studies of different ghost crab species in other regions of the world (see citations in Schalacher et al. 2016a). For conservation and management purposes, it is vital to have empirical biological data to make informed planning decisions (Solomon et al. 2003). For sandy beaches, these data are often obtained through the study of ghost crabs (Schalacher et al. 2016a and citations). We also present the first evidence that human disturbance impacts the sex-ratio of Atlantic Ghost Crabs. Further research is required to understand the consequences of the shift in this ratio and to understand the mechanism of anthropogenic impacts causing a decline in the Atlantic Ghost Crab population density and size structure on sandy beaches.

Overall, in both marine and terrestrial systems, human disturbances have been increasing, and coping with those disturbances may be a serious challenge for their conservation and management. Every ecosystem is unique; thus, studies such as this one, which determine an area-specific bioindicator species (Solomon et al. 2003), are necessary for reliable and accurate management. We have shown

that, the Atlantic Ghost Crab is a viable bioindicator species for sandy beaches in South Carolina that allows for examination of human impacts with relatively low cost and effort.

## **2.7 Acknowledgments**

We thank the staff of Baruch Marine Field Laboratory and Tom Yawkey Wildlife Center for their help. We extend special thanks to Esra Erdil Gül for her great help during data collection.

## 2.8 Tables

Table 2.1. Summary of Generalized Linear Mixed Effects Models showing the outcomes of the best models explaining the changes of burrow diameter (mm) and burrow density (burrow/m<sup>2</sup>) compared to pristine sites (HV: heavily impacted by people and vehicles, HI: heavily impacted by only people, MI: moderately impacted by only people). Sampling days and transects nested within sites were random factors. \* indicates the significant values.

Burrow diameter					Burrow density				
Fixed factor	Coef.	S.E.	t-value	p-value	Fixed factor	Coef.	S.E.	Z-value	p-value
<i>MI</i>	-9.91	3.57	-2.77	0.016*	<i>MI</i>	-0.45	0.13	-3.24	0.001*
<i>HI</i>	-42.91	10.67	-4.01	0.001*	<i>HI</i>	-0.78	0.18	-4.13	<0.001*
<i>HV</i>	-28.62	7.88	-3.63	0.002*	<i>HV</i>	-1.41	0.24	-5.8	<0.001*
<i>Compaction</i>	193.01	93.51	2.06	0.06	<i>Compaction</i>	1.32	2.98	0.44	0.65
<i>Grain size</i>	-19.89	83.14	-0.23	0.81	<i>Grain size</i>	1.19	1.11	0.17	0.86
<i>Latitude</i>	14.25	3.72	3.82	0.004*	<i>Latitude</i>	0.04	0.1	0.45	0.64
<i>MI X grain size</i>	5.82	86.36	0.067	0.94					
<i>HI X grain size</i>	789.54	251.79	3.13	0.008*					
<i>HV X grain size</i>	-131.79	124.17	-1.06	0.301					

## 2.9 Figures

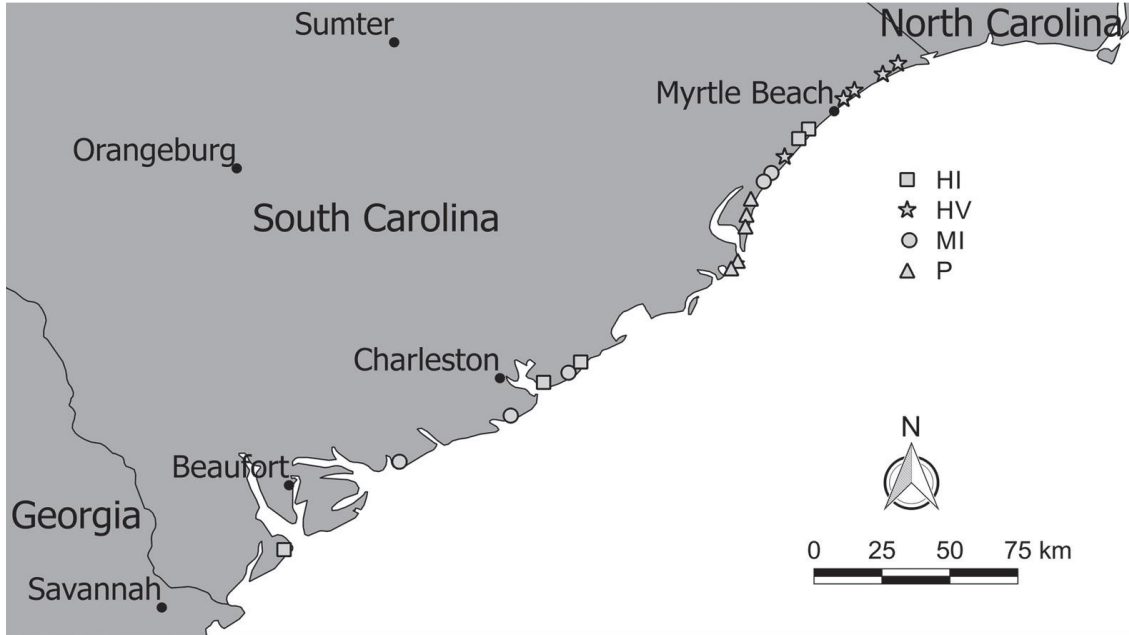


Figure 2.1. Location of the study sites. HV = heavily impacted by people and vehicles, HI = heavily impacted by only people, MI = moderately impacted only by people, and P = pristine). We examined a total of 20 sites. Each human impact type is represented by 5 sites as shown on the map.

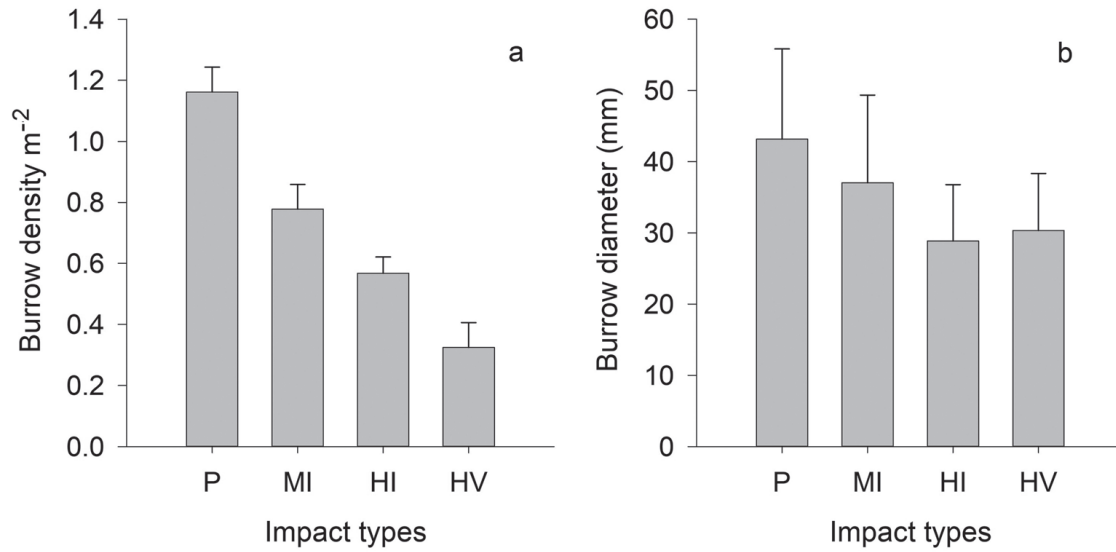


Figure 2.2. Burrow measurements under different impact types. HV = heavily impacted by people and vehicles, HI = heavily impacted only by people, MI = moderately impacted only by people, and P = pristine. (a) burrow density (burrow/ $\text{m}^2 \pm \text{SD}$ ) among impact types. The burrow density declines steadily with increasing disturbance. (b) Burrow diameter ( $\text{mm} \pm \text{SD}$ ) showed a similar, but weaker trend with burrow density, declining with increasing disturbance.

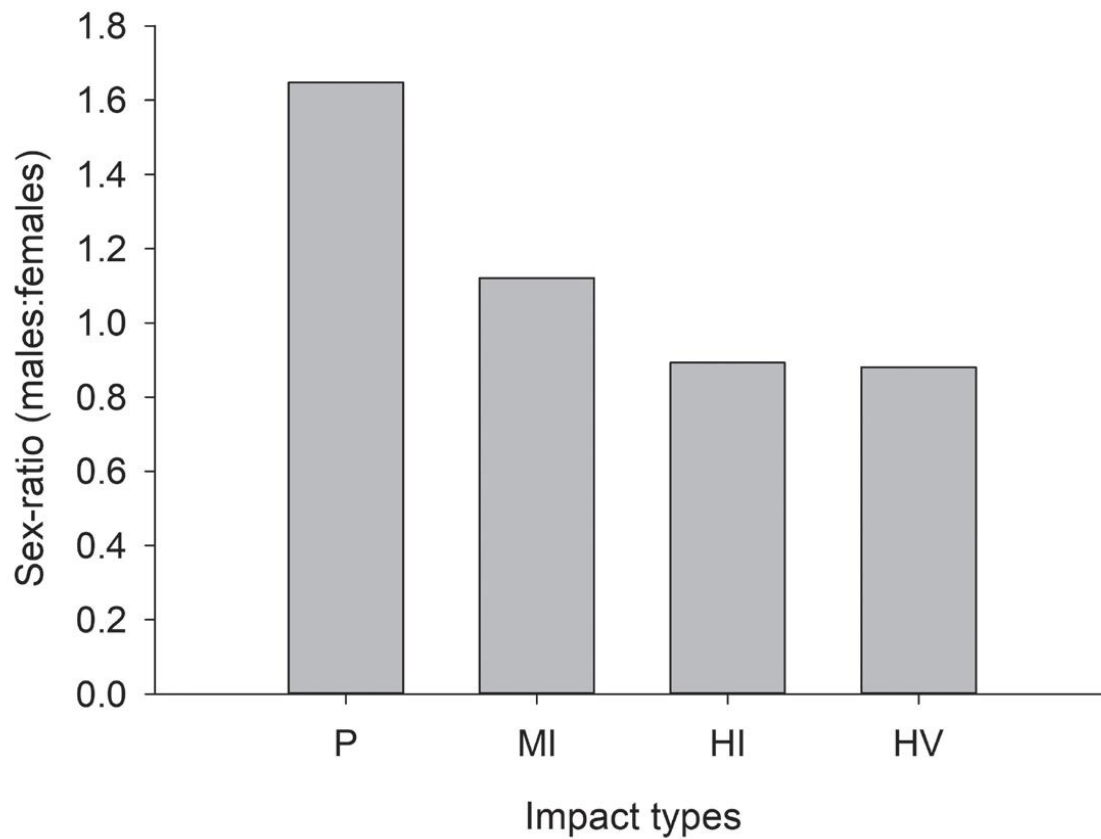


Figure 2.3. Change in the sex-ratio (males:females) of Atlantic ghost crab among impact types (HV: heavily impacted by people and vehicles, HI: heavily impacted by only people, MI: moderately impacted by only people, P: pristine). Females outnumbered males with increasing disturbance. The sex-ratio was similar between pristine and moderately impacted sites, and between the sites highly impacted by only humans and highly impacted by human and vehicles.

## 2.10 Literature Cited

- Barbier, E.B., S.D. Hacker, C. Kennedy, E.W. Koch, A.C. Stier, & Silliman, B.R. (2011). The value of estuarine and coastal-ecosystem services. *Ecological Monographs*, 81(2), 169–193.
- Burnham, K.P., & Anderson, D.R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd Edition. Springer-Verlag, New York, NY. 515 pp.
- Cardoso, R.S., C.A.M. Barboza, V.B. Skinner, & Cabrini, T.M.B. (2016). Crustaceans as ecological indicators of metropolitan sandy-beach health. *Ecological Indicators*, 62, 154–162.
- Carignan, V., & Villard, M.A. (2002). Selecting indicator species to monitor ecological integrity: A review. *Environmental Monitoring and Assessment*, 78(1), 45–61.
- Cheetham, M.D., A.F. Keene, R.T. Bush, L.A. Sullivan, & Erskine, W.D. (2008). A comparison of grain-size analysis methods for sand-dominated fluvial sediments. *Sedimentology*, 55, 1905–1913.
- Cortes, R.M.V., S.J. Hugher, V.R. Pereira, & Varandas, S.G.P. (2013). Tools for bioindicator assessment in rivers: The importance of spatial scale, land-use patterns, and biotic integration. *Ecological Indicators*, 34, 460–477.
- Davenport, J., & Davenport, J.L. (2006). The impact of tourism and personal-leisure transport on coastal environments: A review. *Estuarine, Coastal, and Shelf Science*, 67, 280–292.



- Defeo, O., & A. de Alava, A. (1995). Effects of human activities on long-term trends in sandy beach populations: The Wedge Clam, *Donax hanleyanus*, in Uruguay. *Marine Ecology Progress Series*, 123, 72–82.
- Defeo, O., & A. McLachlan, A. (2011). Coupling between macrofauna community structure and beach type: A deconstructive meta-analysis. *Marine Ecology Progress Series*, 433, 29–41.
- Fisher, J., & Tevesz, M. (1979). Within-habitat spatial patterns of *Ocypode quadrata* (Fabricius) (Decapoda Brachyura). *Crustaceana*, 5, 31–36.
- Folk, R.L. (1980). *Petrology of Sedimentary Rocks*. Hemphill Publishing Company, Austin, TX. 182 pp.
- González, S.A., K. Yáñez-Navea, & Muñoz, M. (2014). Effect of coastal urbanization on sandy beach coleoptera *Phaleria maculate* (Kulzer, 1959) in northern Chile. *Marine Pollution Bulletin*, 83(1), 265–274.
- Hobbs, C.H., C.B. Landry, & Perry, J.E. (2008). Assessing anthropogenic and natural impacts on Ghost Crabs (*Ocypode quadrata*) at Cape Hatteras National Seashore, North Carolina. *Journal of Coastal Research*, 24(6), 1450–1458.
- Maccarone, A.D., & Mathews, P.L. (2007). Effect of human disturbance on the abundance and spatial distribution of the Atlantic Ghost Crab (*Ocypode quadrata*) (Fabricius 1798) on a Texas beach. *The Texas Journal of Science*, 59, 51–60.
- McLachlan, A., O. Defeo, E. Jaramillo, & Short, A.D. (2013). Sandy-beach conservation and recreation: Guidelines for optimizing management strategies for multi-purpose use. *Ocean and Coastal Management*, 71, 256–268.

- Milne, L.J., & M.J. Milne, M.J. (1946). Notes on the behavior of the Ghost Crab. *American Naturalist*, 80, 362–380.
- Neves, F.M., & Bemvenuti, C.E. 2006. The ghost crab *Ocypode quadrata* (Fabricius, 1787) as a potential indicator of anthropogenic impact along the Rio Grande do Sul coast, Brasil. *Biological Conservation*, 133(4), 431–435.
- Pérez-García, J.M., E. Sebastián-González, F. Botella, & Sánchez-Zapata, J. (2016). Selecting indicator species of infrastructure impacts using network analysis and biological traits: Bird electrocution and power lines. *Ecological Indicators*, 60, 428–433.
- Pombo, M., & Turra, A. (2013). Issues to be considered in counting burrows as a measure of Atlantic Ghost Crab populations, an important bioindicator of sandy beaches. *PLoS ONE*, 8(12):e83792.
- R Core Team. 2016 R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Schaleher, T.A., L.M.C. Thompson, & Walker, S.J. (2008). Mortalities caused by off-road vehicles (ORVs) to a key member of sandy-beach assemblages, the Surf Clam, *Donax deltoids*. *Hydrobiologia*, 610, 345–350.
- Schlacher, T.A., S. Lucrezi, R.M. Connolly, C.H. Peterson, B.L. Gilby, B. Maslo, A.D. Olds, S.J. Walker, J.X. Leon, J.M. Huijbers, M.A. Weston, A. Turra, G.A. Hyndes, R.A. Holt, & Schoeman, D.S. (2016a). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal, and Shelf Science*, 169, 56–73.
- Schalacher, T.A., S. Lucrezi, C.H. Peterson, R.M. Conolly, A.D. Olds, F. Althaus, G.A. Hyndes, B. Maslo, B.L. Gilby, J.X. Leo, M.A. Weston, M. Lastra, A. Williams, &

- Schoeman, D.S. (2016b). Estimating animal populations and body sizes from burrows: Marine ecologists have their heads buried in the sand. *Journal of Sea Research*, 112, 55–64.
- Sheppard, N., K.A. Pitt, & Schlacher, T.A. (2009). Sub-lethal effects of off-road vehicles (ORVs) on Surf Clams on sandy beaches. *Journal of Experimental Marine Biology and Ecology*, 380, 113–118.
- Siddig, A.A.H., A.M. Ellison, A. Ochs, C. Villar-Leeman, & Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in ecological indicators. *Ecological Indicators*, 60, 223–230.
- Solomon, M., A.S.V. Jaarsveld, H.C. Biggs, & Knight, N.H. (2003). Conservation targets for viable species assemblages? *Biodiversity and Conservation*, 12, 2435–2441.
- Vitousek, P.M., H.A. Mooney, J. Lubchenco, & Melillo, J.M. (1997). Human domination of Earth's ecosystems. *Science*, 277, 494–499.
- Williams, A.B. (1984). *Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida*. Smithsonian Institution Press, Washington, DC. 550 pp.
- Wolcott, T.G. (1978). Ecological role of Ghost Crabs, *Ocypode quadrata* (Fabricius), on an ocean beach: Scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31(1), 67–82.
- Wolcott, T.G., & Wolcott, D.L. (1984). Impact of off-road vehicles on macroinvertebrates of a mid-Atlantic beach. *Biological Conservation*, 29, 217–240.

## CHAPTER 3

### IMPACTS OF HUMAN DISTURBANCE ON GHOST CRAB BURROW MORPHOLOGY AND DISTRIBUTION ON SANDY SHORES<sup>1</sup>

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<sup>1</sup>Gül MR. & Griffen BD. 2018. Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores. *PLoS ONE*.13(12): e0209977. Reproduced here with permission of publisher.

### **3.1 Abstract**

Ghost crabs have been widely used as a bio-indicator species of human impacts on sandy beaches to obtain reliable biological data for management and conservation purposes. Ghost crab population densities and individual sizes decline dramatically under human pressure. However, distribution within a beach and the factors that determine this distribution of ghost crabs is still an open question. These factors may provide valuable information for understanding human impacts on sandy beaches. Here we examine ghost crab burrows on 20 sandy beaches of South Carolina, USA under various levels of human impacts to understand the response in terms of spatial distribution of this species to human impacts. We also examine the burrow characteristics and environmental properties of the burrows to determine whether these factors alter burrow characteristics. We show that crabs on heavily impacted beaches altered their spatial distribution to mostly occupy the edges of impacted beaches. Further, this change in spatial distribution was influenced by the size distribution of the population on a beach (i.e. larger individuals occupy upper parts on the beaches). We also found that ghost crabs altered the morphology of their burrows on heavily impacted beaches. Ghost crabs create deeper, steeper and smaller burrows under human impacts. These patterns were also influenced by physical characteristics of the beach. Our results suggest that human impacts can directly influence the spatial distribution of ghost crab populations within a beach and therefore sampling at upper parts of the beaches overestimates the population density and individual sizes. Our results support the use of ghost crabs as indicator species in effective beach management, but suggest that assessments would benefit from examining

the morphology and distribution of burrows as opposed to simply using burrow counts to assess the health of sandy shores.

### **3.2 Introduction**

Human impacts have become the major force shaping ecosystems globally over the past century. The strength of these impacts in different regions are largely correlated with human population size and changes in the land-use dynamics (Ojima et al. 1994). Currently, human populations in coastal areas are growing faster than in other non-coastal regions (Davenport & Davenport 2006).

Coastal ecosystems are highly preferred regions due to their valuable services (Davenport & Davenport 2006, McLachlan & Brown 2006) such as recreation and tourism (Davenport & Davenport 2006, Wolcott & Wolcott 1984). While recreation and tourism provide a socio-economic boost (James 2000, Schlacher et al. 2007, Lucrezi & Schlacher 2010), they cause important ecological issues (Wolcott & Wolcott 1984) such as alteration in natural habitats, declines in biodiversity and increase in pollution (Halpern et al. 2008), which can cause either reversible or irreversible damage in coastal ecosystems (McLachlan & Brown 2006, Defeo et al. 2009).

The degree of ecological change in marine ecosystems is strongly correlated with the intensity of human impacts (Halpern et al. 2008). On sandy beaches, those ecological alterations mostly are changes in fauna and flora (Defeo et al. 2009, Steiner & Leatherman 1981, Barros 2001), decreases in abundance and biodiversity (Schlacher et al. 2007, Defeo et al. 2009) and declining ecosystem services (Barbier et al. 2011). While human impacts may alter these aspects of marine ecosystems (Vitousek et al. 1997), the

extent of human impacts is often monitored using bio-indicator species or assemblages (Solomon et al. 2003). Examples include bean clams, *Donax* spp. (Defeo & de Alava 1995, Schlacher et al. 2008, Sheppard et al. 2008), mole crabs, *Emerita* spp. (Cardoso et al. 2016), sand beach coleoptera, *Phaleria* spp. (González et al. 2014) and ghost crabs, *Ocypode* spp. (see Schlacher et al. 2016a and citations) and *Hoplocypode* spp. (Sakai & Türkay, 2016). These species have been used extensively due to their high abundance and sensitivity to human impacts (Carignan & Villard 2002).

Ghost crabs have been widely used as a bio-indicator species to determine the ecological impacts of human use of sandy beaches globally (Schlacher et al. 2016a and citations) because of their strong responses to anthropogenic (Neves & Bemvenuti 2006) and natural impacts (Hobbs et al. 2008), as well as their relatively large size and characteristic behaviors (Barros 2001). Also, their burrowing behavior provides an advantage for a low-cost and efficient monitoring technique (Pombo & Turra 2013) that has been applied to many sandy beaches in various locations around the world such as in North Carolina (Wolcott & Wolcott 1984, Hobbs et al. 2008), Virginia and Maryland (Steiner & Leatherman 1981), Ghana (Aheto et al. 2011, Jonah et al. 2015), Brazil (Neves & Bemvenuti 2006), South Africa (Lucrezi et al. 2014), as well as in Australia (Barros 2001, Lucrezi et al. 2009, Schlacher & Lucrezi 2010, Lucrezi & Schlacher 2009).

While ghost crabs have been broadly used as indicator species, there are still aspects of their ecology and responses to human impacts that we do not understand. These include understanding the gradation in ghost crab responses, both in terms of individual behaviors and in terms of population abundance, across different levels of

human impacts. Developing a better understanding of these factors will enhance the usefulness of ghost crabs as bioindicators of sandy beaches.

The purpose of this study was to investigate how various types and levels of human impacts alter the burrowing characteristics such as burrow volume, depth, inclination angle and shape and population levels like density, distribution and individual sizes of burrows of the Atlantic ghost crab, *Ocypode quadrata*, on South Carolina sandy beaches. We hypothesized that ghost crabs would produce smaller burrows and would be found in lower densities in areas that experienced the most extreme disturbance due to detrimental activities of visitors on sandy shores, and that burrowing properties would be more sensitive than burrow density as a bioindicator metric.

### **3.3 Methods**

#### *3.3.1 Study Sites*

Twenty sandy beaches under various levels of human disturbance located on the coast of South Carolina, United States were sampled between 26th May and 28th September 2016 (Fig. 3.1). The total distance between the northernmost study site (North Myrtle Beach) and southernmost study site (Burkes Beach, Hilton Head) was about 300 km. All field research was conducted under a permit issued by South Carolina Department of Natural Resources (Permit Numbers: 4044, 4261). No protected species were disturbed during our research. All study sites are ocean exposed sandy beaches, however, because of a wide continental shelf, the wave energy affecting the coast of South Carolina is relatively low (Kana 1988).



We divided our study sites in 4 different groups in terms of impact levels based on our observations of intensity of visitors, whether or not beaches experienced mechanical cleaning and presence or absence of the vehicles on the sand. In order to determine the intensity of the visitors on the beaches, we counted the number of people at each site using binoculars over a two-hour period from 0800 to 1000 h over approximately a linear kilometer of the beach during a normal workweek. We observed whether the beach was mechanically cleaned at night by noting both the vehicles and their tire-marks on the sand.

The sites that are not accessible by vehicles were considered as pristine sites, because no pedestrians or vehicles were observed. We considered the beaches as moderately impacted sites when they were visited by less than 50 pedestrians per hour. The number of visitors at moderately impacted sites ranged from 31 people  $\text{h}^{-1}$  at Pawley's Island to 48 people  $\text{h}^{-1}$  at Folly Beach with an average of  $39.3 \pm 17.5$  ( $\pm \text{S.D.}$ ) people  $\text{h}^{-1}$ . On the other hand, the number of visitors at highly impacted sites by only people were between 54 people  $\text{h}^{-1}$  at Surfside Beach and 63.5 people  $\text{h}^{-1}$  at Sullivan's Island with an average of  $60.2 \pm 32.08$  ( $\pm \text{S.D.}$ ) people  $\text{h}^{-1}$ . Finally, the visitor numbers at highly impacted sites by people and vehicles ranged from 58.5 people  $\text{h}^{-1}$  at Garden City Beach to 89.5 people  $\text{h}^{-1}$  at Myrtle Beach 1 with an average of  $72.3 \pm 13.5$  ( $\pm \text{S.D.}$ ) people  $\text{h}^{-1}$ . For a detailed description of the study sites see [33].

### *3.3.2 Spatial burrow distribution*

Gül & Griffen (2018) used an indirect burrow examination technique on South Carolina sandy shores to understand whether ghost crab burrow density and size varied in

relation to presence of human disturbance. They only examined burrows within 2.5 m of the backshore vegetation within each site and thus did not collect any data for spatial burrow distribution and the variation in the burrow density and size at different tidal heights.

In order to determine if the distribution patterns of the ghost crab burrows change due to human disturbance, we observed the burrow opening diameter (hereafter burrow diameter). At each site, we sampled in three replicate rectangular quadrats (10 m X 5 m) at each of five tidal heights parallel to the shore at low tides. Quadrats at the same tidal height within a beach were spaced ~100 m from each other. The first quadrat (T1) was deployed on the seaward site of the dune vegetation. The fourth (T4) and the fifth (T5) quadrats were applied on the landward and seaward sites of the strand line, respectively. Since the beach width varied among study sites, we positioned the second (T2) and third (T3) quadrats between T1 and T4 so that all were separated by approximately equal distances. This meant that the distance between quadrates T1-T4 differed across sites depending on the width of the shore.

In each quadrat, only the active ghost crab burrows were examined. We observed the fresh sand and/or traces left by individuals around the burrow mouths to decide if a burrow was active or not (Lucrezi et al. 2009, Schlacher & Lucrezi 2010, Lucrezi & Schlacher 2009). Since crab size is positively related to its burrow opening diameter in Atlantic ghost crab ( $r^2 = 0.98$ ; Wolcott 1978), we examined ghost crab burrows by measuring the largest distance of the burrow openings as burrow diameter using a Vernier caliper ( $\pm 0.1$  mm) and the distance between the burrow openings and the backshore vegetation (hereafter distance). When the burrow had more than one opening,

we considered only the main opening by observing the traces around it. We used a linear mixed effects model to determine whether the size of ghost crab burrows varied among tidal heights and the beaches that were under various levels of human disturbance. Human impact level was treated as categorical fixed factor. Human impact types and quadrats (i.e. tidal height on the beach) were treated as categorical and ordinal fixed factors, respectively. To control for daily and latitudinal difference, sampling time as julian day and latitude of the sampling site were added as random factors to this analysis and all subsequent statistical analysis in this paper. Also, for this and all subsequent statistical analysis, we used Tukey's multiple comparison test to determine the pairwise differences among impact types. We used the statistical software R version 3.3.2 (R Core Team 2017) for all statistical analyses.

To understand if the burrow density (burrows/m<sup>2</sup>) was affected in different parts of beaches under various impact levels, we counted the number of the active burrows in each quadrat and analyzed these data using a generalized linear mixed effects model (GLMM) with a Poisson distribution. The impact level was treated as fixed factor and the quadrat number (i.e., relative height on the shore) was treated as an ordinal variable.

### *3.3.3 Burrow architecture*

The following procedure was conducted after sunset to make sure that all individuals had already left their burrows. Burrows (1 to 3 burrows from each quadrat level with various opening sizes (except T5 because it was often submerged) were selected randomly. We poured a 2:1 mixture of plaster of Paris and freshwater into the burrows until they were fully filled (Chan et al. 2006). We waited for about 30 minutes

until the plaster was dried, after which we excavated the casts using a shovel. We measured the angle of inclination of the casts relative to horizontal. After excavation, we measured the depths using a ruler from the surface to the deepest point of the casts. We then labeled the casts and took them to the laboratory for further investigations.

In the laboratory, we measured the diameter every three-centimeters along the length of each cast using a Vernier caliper ( $\pm 0.1$  mm) and obtained the average diameter of each cast. The volume of the casts was obtained by using the equation for the volume of cylinder and using the average diameter of each cast. The shapes and the patterns of the casts were obtained by visual observations. We used chi-square to test the variation in burrow shapes between human impact levels.

Burrow size and morphology may also be influenced by physical characteristics of the site. Thus, we measured the surface temperature and moisture (i.e. relative humidity) for 10 minutes every hour during the night using a data logger (Onset U23-001, HOBO Pro v2). We also measured the temperature and moisture—of the burrows at the deepest point just after we excavated the casts. Because these excavations were done at night, we assume that the brief aerial exposure following excavation and before measurement did not substantially influence these measurements; and any influence on temperature or moisture from aerial exposure that did occur would have been similar across all burrows, and therefore would not influence our cross-site comparison.

To obtain geomorphological properties of the study sites, we measured the sand compaction, sand grain size and beach slope. We obtained the sand compaction by using a pocket penetrometer (AMS G281) applied five times in each site. Since the strength of

the sand was very low, we applied the penetrometer with its adaptor foot that increases the surface area of the measurement by 16 times. At the end of the measurements, we divided our results by 16 to obtain the actual compaction values. For grain size, we used the sieving method (Folk 1980). We collected three 500 g samples from each site. The sand was dried and separated through a series of sieves with different mesh sizes (2 mm, 1 mm, 0.5 mm, 0.42 mm, 0.25 mm, 0.177 mm, 0.125 mm and 0.074 mm). Finally, we obtained the slope of the study sites by dividing the elevation difference between the beginning of the backshore vegetation and the strandline by the distance between those two points. For each study site, the same technique was applied three times.

Since the burrow volume and depth are positively correlated with the size of burrow opening (Chan et al. 2006, Trivedi & Vachhrajani 2016), which itself is a proxy of crab size (Wolcott 1978), we regressed log-transformed burrow architectural data against log-transformed burrow diameter to remove the effects of crab size (Packard & Boardman 1999). The standardized residuals from those regressions were used as response variables for all subsequent statistical analyses for burrow architecture. We used linear mixed effects models to examine the factors that influenced the variation in burrow metrics. The residuals of burrow volume and depth, after accounting for crab size, and inclination angle were treated as the response variables in separate analyses. The suite of predictor variables varied depending on the expected relationships for each response variable. For the model examining burrow volume, we used impact level, sand grain size, distance from backshore vegetation, sand compaction and all the possible interactions of those variables as fixed factors. For the model examining burrow depth, we used burrow temperature and humidity in addition to the variables listed above. Random factors were

the standard factors described above for all analyses in this study. For each of these analyses, we selected the best fitting models using the lowest AICc values (dredge function in MuMIn package in R, Bartoń 2017). When more than one model with  $\Delta\text{AICc} < 2$  existed, the models were averaged (Burnham & Anderson 2002).

### **3.4 Results**

#### *3.4.1 Burrow size and distance from backshore vegetation*

A total of 5565 ghost crab burrows were examined in terms of their size, density and the distance from vegetation. Burrow diameter showed a notable difference in the sites that were heavily disturbed compared to less impacted sites (Fig. 3.2). The burrow diameter in pristine sites was significantly higher than the burrow diameter in the sites that were moderately impacted (LMER, estimate = -7.39,  $z = -7.11$ ,  $p < 0.001$ ), highly impacted by people (LMER, estimate = -10.74,  $z = -10.03$ ,  $p < 0.001$ ) and highly impacted sites by people and vehicles (LMER, estimate = -15.26,  $z = -13.33$ ,  $p < 0.001$ ). Unsurprisingly, a higher average of burrow diameter was found in moderately impacted sites compared to highly impacted sites by people (LMER, estimate = -3.35,  $z = -3.28$ ,  $p = 0.005$ ) and highly impacted sites by people and vehicles (LMER, estimate = -7.87,  $z = -7.15$ ,  $p < 0.001$ ). Also, presence of vehicles on the sandy shores caused a lower burrow diameter in the sites that are heavily disturbed (LMER, estimate = -4.51,  $z = -3.39$ ,  $p < 0.001$ , Fig. 3.2)

We found zonation in burrow size and location on the beaches in all disturbance levels (Fig. 3.3). The results of LME indicated that there was clear difference in the zonation of burrow sizes in pristine sites compared to moderately impacted sites (LME,

estimate = 2.53,  $t = 11.16$ ,  $p < 0.001$ ) and highly impacted sites (by people: LME, estimate = 2.53,  $t = 14.29$ ,  $p < 0.001$  and by people and vehicles: LME, estimate 4.71,  $t = 14.78$ ,  $p < 0.001$ ). Random factors were as follows: latitude variance component = 0.275 (S.E. = 0.524) and Julian days variance component = 1.013 (S.E. = 1.006).

### *3.4.2 Burrow density*

We found that the zonation of ghost crab burrows was altered by human impacts. While, the burrow density across beaches in pristine sites were similar to each other, ghost crabs mostly occupied the edges of the sites (upper and lower parts of beaches) under human impacts. The ghost crab burrow density was significantly lower in the sites that were under human impacts compared to pristine sites, and also differed among quadrats (Table 3.1, Fig. 3.4). Expectedly, burrow density was lower in moderately impacted (estimate = -0.68,  $z = -5.13$ ,  $p < 0.001$ ), highly impacted by people (estimate = -1.15,  $z = -8$ ,  $p < 0.001$ ) and highly impacted by people and vehicles (estimate = -1.88,  $z = -11.6$ ,  $p < 0.001$ ) sites compared to the burrow density in pristine sites. Both highly impacted sites by people (estimate = -0.47,  $z = -3.27$ ,  $p = 0.0058$ ) and highly impacted sites by people and vehicles (estimate = -1.2,  $z = -7.39$ ,  $p < 0.001$ ) had lower burrow densities than moderately impacted sites. Additionally, vehicle impacts on sandy shores caused a lower burrow density (highly impacted sites by people vs highly impacted sites by people and vehicles: estimate = -0.73,  $z = -4.3$ ,  $p < 0.001$ , Fig. 3.2).

### *3.4.3 Burrow architecture and metrics*

We examined a total 146 ghost crab burrow casts of seven shapes resembling the letters I, Y, L, J, U, V and M. Regardless of impact level, most of the burrows were of I

(n = 47) with 32.87% and Y (n = 39) with 26.71% shapes (Fig. 3.5). Burrows with single-opening shapes (I, J, L) were more common (55.47%) than more complex-shaped with multi-openings burrows (Y, U, V, M) (44.52%). Burrow shapes in pristine sites differed significantly compared to burrow shapes in moderately impacted sites (Chi-squared test,  $X^2 = 418.4$ ,  $df = 42$ ,  $p < 0.001$ ) and in heavily impacted sites (by people: Chi-squared test,  $X^2 = 438$ ,  $df = 49$ ,  $p < 0.001$  and by people and vehicles: Chi-squared test,  $X^2 = 303.26$ ,  $df = 49$ ,  $p < 0.001$ ). In addition, burrow shapes in moderately impacted sites were different from the burrow shapes in highly impacted sites by people (Chi-squared test,  $X^2 = 531.33$ ,  $df = 42$ ,  $p < 0.001$ ) and in highly impacted sites by people and vehicles (Chi-squared test,  $X^2 = 330.54$ ,  $df = 42$ ,  $p < 0.001$ ). Burrow shapes also changed with the impact of vehicles in heavily impacted sites (Chi-squared test,  $X^2 = 527.53$ ,  $df = 49$ ,  $p < 0.001$ ). Unlike the previous studies on different ghost crab species [31], we found no chambers at any parts of the burrows.

Metrics of the burrow casts clearly varied among sites based on impact level. Specifically, ghost crab burrow volume was similar between heavy impacted beaches (highly impacted site by people vs highly impacted sites by people and vehicles: LMER,  $z = -1.03$ ,  $p = 0.69$ ) and also between slightly impacted beaches (moderately impacted sites vs pristine sites: LMER,  $z = -2.4$ ,  $p = 0.065$ ). Additionally, ghost crabs appeared to create larger burrows in pristine sites compared to highly impacted sites by people (LMER,  $z = -3.12$ ,  $p = 0.008$ ) and highly impacted sites by people and vehicles (LMER,  $z = -3.27$ ,  $p = 0.004$ ). Surprisingly, there was no significant difference between moderately impacted sites and highly impacted sites by people (LMER,  $z = -2.32$ ,  $p = 0.08$ ) in terms of burrow volume (Table 3.2, Fig. 3.6). According to the best fitting models,



geomorphological properties of the sandy beaches (i.e. sand compaction and grain size) were correlated with the burrow volume (Table 3.2). The average sand compaction (range: 0.04–0.131 kg cm<sup>-2</sup>) and grain size (range: 0.238–0.52 mm) were 0.086 kg cm<sup>-2</sup> and 0.32 mm, respectively.

Ghost crabs appeared to dig deeper burrows as the level of human impact increased. Ghost crab burrows were shallower in pristine sites compared to moderately impacted sites ( $z = 1.87$ ,  $p = 0.023$ ), highly impacted sites by people ( $z = 3.15$ ,  $p = 0.008$ ) and highly impacted sites by people and vehicles ( $z = 3.92$ ,  $p < 0.001$ ). However, burrow depths were similar in moderately impacted and highly impacted sites by people ( $z = 1.66$ ,  $p = 0.33$ ), the depth was higher in highly impacted sites by people and vehicles ( $z = 2.72$ ,  $p = 0.032$ ). Also, there was no difference in burrow depth between highly impacted sites by only people and by people and vehicles together ( $z = 1.12$ ,  $p = 0.67$ , Table 3.2, Fig. 3.6). Moreover, inclination angle became steeper with increasing human impact and sand grain size and the distance from the backshore vegetation. Ghost crab burrows were steeper in highly impacted sites by people and vehicles compared to burrows in pristine ( $z = -9.15$ ,  $p < 0.001$ ), moderately impacted sites ( $z = -4.81$ ,  $p < 0.001$ ) and highly impacted sites by people ( $z = -4.68$ ,  $p < 0.001$ ). The inclination angle of the ghost burrows was lower in both moderately impacted ( $z = -4.87$ ,  $p < 0.001$ ) and highly impacted sites by people ( $z = -5.04$ ,  $p < 0.001$ ) compared the burrows in pristine sites (Table 3.2, Fig. 3.6). The best fitting model showed that burrow inside temperature was correlated with burrow depth, but relative humidity was not (Table 3.2, Fig. 3.7).

### 3.5 Discussion

We have shown that the distribution of the *O. quadarata* burrows on sandy beaches vary among the sites that are under influence of various levels and the types of human disturbance. We have further demonstrated that *O. quadrata* populations responds strongly to various human impact types by declining in both size and density, and burrowing characteristics and burrow dimensions vary based on human impacts on sandy beaches.

Previous studies have shown that environmental variables can also influence ghost crab burrow density, size and architecture such as temperature, moisture level, wind (Lucrezi et al. 2009), and geomorphological properties of the sandy shores like sand compaction, beach slope and sand grain size (Dixon et al. 2015, Pombo et al. 2017) and as well as erosion (Hobbs et al. 2008). Since the main goal of this work was to understand the impact of human disturbance, we did not examine these other factors.

#### 3.5.1 Burrow density and size

The results of our study reflect that the overall density and the average burrow size dramatically decline at the sites where human impacts exist. Several studies have shown that vehicles (Wolcott & Wolcott 1984, Steiner Leatherman 1981, Hobbs et al. 2008, Schlacher et al. 2008, Lucrezi & Schlacher 2009, Lucrezi et al. 2009, Lucrezi & Schlacher 2010, Schlacher & Lucrezi 2010, Lucrezi et al. 2014), intense pedestrian trampling (Neves & Bemvenuti 2006, Lucrezi et al. 2009) and cold fronts (de Souza et al. 2017) cause a significant decline in number and size in ghost crab populations and our findings in terms of reductions in ghost crab populations are consistent with those studies.

It has been found that individuals using burrows that are shallower than 25 cm are likely to be crushed by vehicles (Schlacher et al. 2007). This finding may explain the reason why the density is low at the sites where vehicles are allowed, especially when they are allowed during the day time when crabs are in their burrows. It seems reasonable to explain the decline in the individual crab size under vehicles disturbance by considering the direct crushing by vehicles during night times (Wolcott & Wolcott 1984), since the larger individuals are slower than the middle size ones (Burrows & Hoyle 1973, Blickhan & Full 1987) and so they may not be fast enough to escape vehicles during night time activity. But, these arguments cannot explain the decline in the ghost crab populations in the sites where the vehicles are not present. During our sampling, we observed people catching and playing with crabs during night times. They mostly catch the larger individuals, possibly because they are easier to see and catch. Stress from this type of human encounter may contribute to the decline in density and size for those sites that have intense human impact.

Changes in food abundance may also contribute to ghost crab declines. Common prey items including bean clams (*Donax* spp.), mole crabs (*Emerita* spp.) and sandy beach coleoptera (*Phaleria* spp.) decline dramatically due to human disturbance (Schlacher et al. 2008, Sheppard et al. 2008, González et al. 2014, Cardoso et al. 2016). This decline in food items may alter energy dynamics of ghost crabs in ways that limit growth rate. Reductions in bean clams and mole crabs may be especially problematic since they are the most preferred food items given their high caloric content (Wolcott 1978). If this is the case, then smaller individuals in highly disturbed sites may potentially be similar ages as larger individuals in pristine and moderately impacted sites, reflecting

lower growth rates due to food limitation. Overall, the reason for the reductions in the density and the individual size of ghost crab populations is still not clear (Schlacher et al. 2007) and most probably cannot be explained by using a single mechanism. Further research is required to investigate the possible reductions in the growth rate of ghost crabs related to food availability in highly disturbed sites.

Additionally, it is possible that the number of burrows (i.e. territory size) differs for crabs on beaches with different levels of human impact (Silva & Calado 2013). If so, burrow counts could cause spurious estimates of crab density across beaches. But, it should not change the overall results of the burrow size, since individuals excavate burrows relative their carapace width (Wolcott 1978). Moreover, a higher mortality or injury rate for larger individuals on the drift zones in unprotected beaches due to cold fronts may contribute to the lower density (de Souza et al. 2017).

### *3.5.2 Spatial distribution of ghost crab burrows*

Our findings demonstrate that the burrow density is relatively homogenous across the pristine and moderately impacted beaches, but that individuals mostly prefer the edges of the beaches for burrowing in highly disturbed sites. Also, smaller individuals mostly occupy the landward areas of the strandline and the upper intertidal areas in highly disturbed sites. Contrary to this, smaller individuals (even juveniles) are more evenly dispersed across the shore in pristine and moderately impacted sites. Our findings in terms of lower moisture content at the sites where a high level of human impact exists are consistent with Steiner & Leatherman (1981) and Lucrezi & Schlacher (2010) probably due to the high compaction rate. As semi-terrestrial organisms, ghost crabs can burrow in both dry and wet areas (Fisher & Tevesz 1979) but always need to moisten

their gill chambers for respiration (Wolcott 1976). Also, the frequency of moistening their gill chambers varies based on size (i.e. juveniles require more frequent replenishment of the water in their gill chamber than adults) (Wolcott 1976). Thus, finding the smaller burrows next to the strandline seems reasonable, since smaller individuals are not capable of digging as deep burrows (Chan et al. 2006) to access the sufficient moisture content in the sand (Wolcott 1976). Also, reductions in the inclination angle and the increase in size-independent burrow depth with increasing impact level might be evidence for the burrowing behavior of accessing an area of higher moisture content. While these arguments seem reasonable, they do not explain the fact that juveniles were found dispersed across all portions of pristine beaches. This finding could therefore indicate that juveniles are not required to renew the water in their gill chamber as often as anticipated, or that they spend more time outside of their burrows and access water during day time (Wolcott 1976) in pristine and moderately impacted sites.

Biological reasons could also explain the distribution patterns of the individuals in various sizes. According to our findings here, in highly impacted sites, mostly smaller individuals occupy the areas closer to strandline and larger individuals occupy the areas closer to dunes that are more stable parts of the beaches. The higher density next to the backshore vegetation may be due to less impact by cleaners especially in the beaches that are mechanically cleaned. We have observed that the cleaners do not approach the vegetation, leading to less frequent disturbance in that part of the beach. Thus, the distribution may reflect the presence of vegetation. It seems reasonable to conclude that larger individuals competitively exclude smaller ones from those more stable portions of the beach. More studies are required to investigate the biological influences on the

distribution of the ghost crabs under various impact levels and types such as intraspecific competition for space.

Ghost crabs are relatively intolerant to cold temperatures because of the associated low humidity (Milne & Milne 1941). However, while the moisture pattern of the ghost crab burrows has been studied, it remains unclear whether burrow temperature plays any role on the cross-shore distribution of ghost crab burrows (Rodrigues et al. 2016). Our results show that *O. quadrata* burrow characteristics are correlated with burrow moisture content, suggesting that burrow humidity may be an important factor. In highly disturbed sites, crabs dig deeper burrows, perhaps to reach more humid sediments. We have also shown that the temperature inside of *O. quadrata* burrows is not as important as moisture level for determining burrow morphology. These findings suggest that regardless of life stage and the position on the beach, ghost crabs require similar amount of moisture and they can tolerate variations in temperature in their burrows.

### 3.5.3 Burrow architecture and metrics

We expected to find simpler burrow construction in highly disturbed sites especially when the site is impacted by the vehicles because of higher energy requirements of the complex-shaped burrows (Lucrezi & Schlacher 2010). We instead found that *O. quadrata* create similarly shaped burrows under all types and levels of human impacts, although the proportion of the complex-shaped (Y, U, V, M) burrows varied among the level of human impacts. Higher proportion of the burrows with second arm could be a strategy of escaping from the potential predators or water entry from wave splash (Chakrabarti 1981). Since we have found no relation between burrow shapes and

location within a beach, it is more likely that individuals create those complex-shaped burrows as a precaution against predators, or cannibals, rather than water entry.

Our finding of simple burrow construction is consistent with previous reports for this species (Silva & Calado 2014), and may be an energy saving strategy (Lucrezi & Schlacher 2010). The smaller and deeper burrows in highly impacted sites by people and vehicles support this idea. Physical pressure exerted by the weight of vehicles is greater than that produced by pedestrians and thus should reach deeper points. Thus, individuals excavate deeper, smaller and simpler burrows at highly impacted sites by people and vehicles both to save energy while still attaining protection and reaching the appropriate moisture.

### **3.6 Conclusion**

We demonstrate that *O. quadrata* alters the density, individual size, burrowing behavior and type and distribution patterns with increasing disturbance on sandy shores. These strong responses to even a small change in human disturbance makes *O. quadrata* a highly reliable bio-indicator species to determine and monitor the ecological consequences of human impacts on sandy beaches. Coastal managers often require ecological data to set up successful conservation plans. As a simple tool, burrow counts have been used previously (see Schlacher et al. 2016) as a reliable indicator of population density and dynamics and individual sizes (Wolcott 1978, de Oliveira et al. 2016, Schlacher et al. 2016b, Suciu et al. 2018). As mentioned by Defeo & Rueda (2002) for density of sandy beach organisms, we suggest cross-shore sampling to determine the variations in population structure, burrowing behavior and distribution pattern, all of

which can increase the strength of the data collected for management purposes. We further suggest that combining the abundance and morphology data of ghost crab burrows for determining the health of sandy shores is a promising and low-cost technique for coastal managers.

### **3.7 Acknowledgments**

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### **3.8 Author Contributions**

**Conceptualization:** Mustafa R. Gül, Blaine D. Griffen.

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**Project administration:** Mustafa R. Gül.

**Resources:** Mustafa R. Gül, Blaine D. Griffen.

**Software:** Mustafa R. Gül.

**Supervision:** Mustafa R. Gül, Blaine D. Griffen.



**Validation:** Mustafa R. Gül, Blaine D. Griffen.

**Visualization:** Mustafa R. Gül.

**Writing – original draft:** Mustafa R. Gül.

**Writing – review & editing:** Mustafa R. Gül, Blaine D. Griffen

### 3.9 Tables

Table 3.1. Summary of Generalized Linear Mixed Effects Model (GLMM) with Poisson distribution showing the burrow density (burrow/m<sup>2</sup>) in quadrats and sites under various types of human impacts compared to pristine sites. \* indicates the significant values.

fixed factor	S.E.	Z-value	p-value
<b>A (moderate impact)</b>	0.136	-5.01	<0.001*
<b>B (high impact by only people)</b>	0.144	-8.01	<0.001*
<b>C (high impact by people and vehicle)</b>	0.162	-11.6	<0.001*
<b>D (quadrat)</b>	0.011	0.67	0.49
<b>A x D</b>	0.026	-8.89	<0.001*
<b>B x D</b>	0.030	-6.28	<0.001*
<b>C x D</b>	0.037	-3.03	<0.001*
<b>Random factor</b>	Variance component		S.E.
<b>Latitude</b>	<0.001		<0.001
<b>Julian days</b>	0.022		0.151

Table 3.2. Summary of Linear Mixed Effects Model (LMER) with a Gaussian distribution showing the residual burrow metrics (burrow volume (cm<sup>3</sup>), burrow depth (cm) and burrow inclination angle (°) of ghost crab populations after accounting for crab size in the sites under various types of human impacts compared to pristine sites.

<b>Burrow volume</b>			
<b>fixed factor</b>	<b>S.E.</b>	<b>t-value</b>	<b>p-value</b>
<b>A (moderate impact)</b>	0.075	-2.4	0.029*
<b>B (high impact by only people)</b>	0.094	-3.12	0.006*
<b>C (high impact by people and vehicle)</b>	0.104	-3.27	0.004*
<b>D (compaction)</b>	6.142	2	0.063
<b>E (grain size)</b>	1.875	2.21	0.04*
<b>D x E (grain size x compaction)</b>	18.181	-1.95	0.067
<b>Random factor</b>	Variance component		S.E.
<i>Julian days</i>	<0.001		<0.001
<i>Latitude</i>	<0.001		<0.001
<b>Burrow depth</b>			
<b>A</b>	0.028	2.12	0.033*
<b>B</b>	0.03	3.79	0.0001*
<b>C</b>	0.029	5.086	<0.0001*
<b>F (humidity)</b>	0.057	0.002	0.99
<b>G (temperature)</b>	0.047	2.23	0.025*
<b>F x G</b>	0.021	0.002	0.99
<b>Random factor</b>	Variance component		S.E.
<i>Julian days</i>	0.0005		0.023
<i>Latitude</i>	0.0045		0.094
<b>Inclination angle</b>			
<b>A</b>	2.72	-4.87	0.0008*
<b>B</b>	2.65	-5.04	0.0006*
<b>C</b>	2.75	-9.15	<0.0001*
<b>E</b>	14.8	0.94	0.36
<b>H (distance)</b>	0.104	-1.57	0.11
<b>Random factor</b>	Variance component		S.E.
<i>Julian days</i>	<0.001		<0.001
<i>Latitude</i>	7.306		2.703

### 3.10 Figures

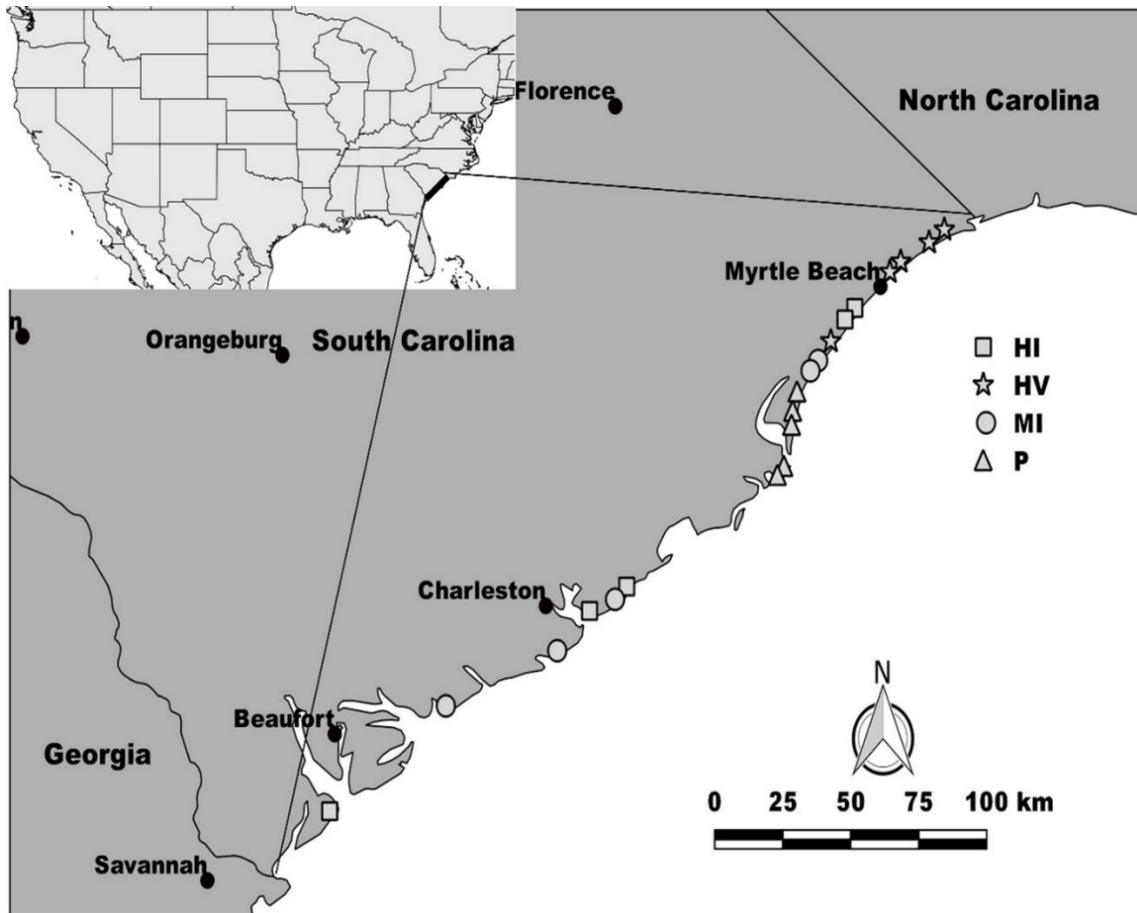


Figure 3.1. Study sites. In the map, P represents the pristine sites, MI represents the moderately impacted sites by only people, HI represents the highly impacted sites by only people and HV represents the highly impacted sites by people and vehicles. Map was created with QGIS v3.0 software

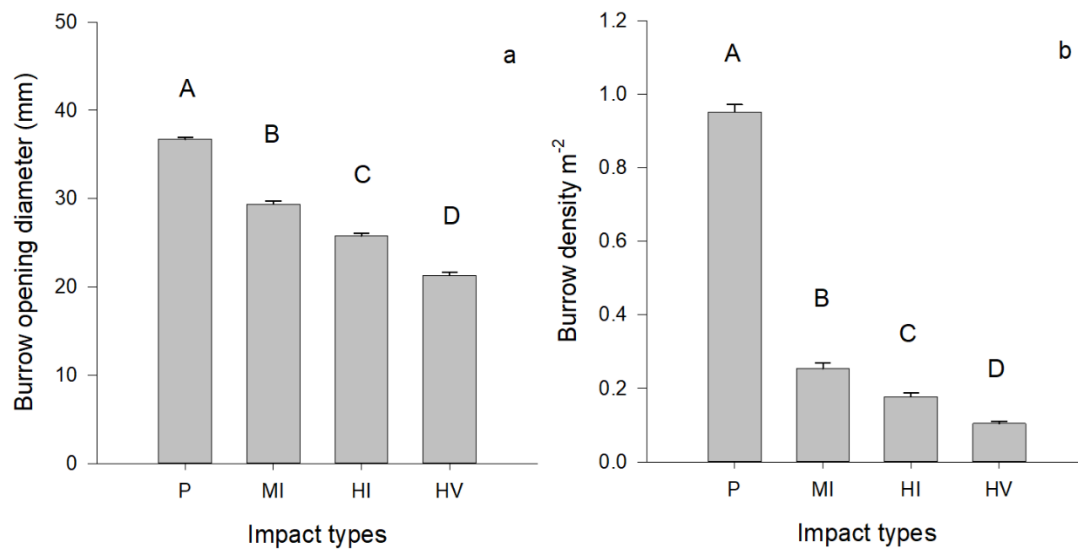


Figure 3.2. Overall ghost crab burrow density and opening diameter among impact types. Variation in burrow density (mean burrow/  $m^{-2} \pm S.E.$ ) (a) and burrow opening diameter (mm  $\pm S.E.$ ) (b) for *O. quadrata* among impact types on South Carolina beaches. (P = pristine sites, MI = moderately impacted sites, HI = highly impacted sites by only people, HV = highly impacted sites by people and vehicles). Letters above bars represent significant differences

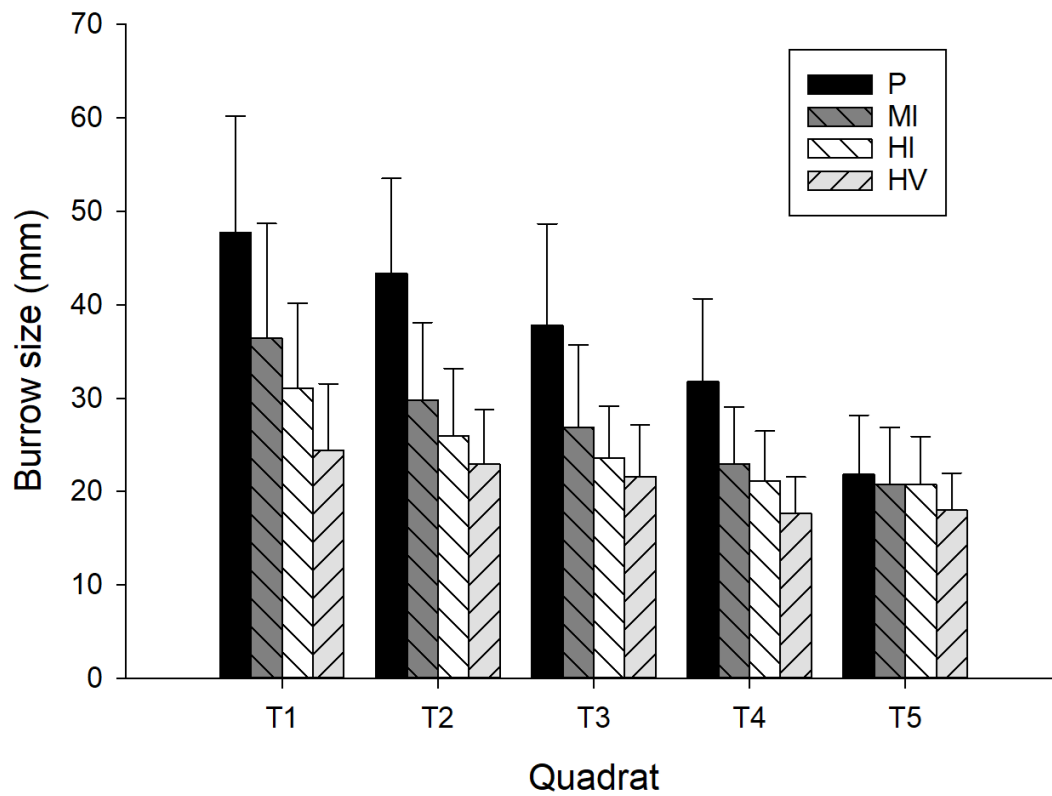


Figure 3.3. Ghost crab burrow diameters among impact types. Relationship between the diameter (mm) of *O. quadrata* burrows and the distance from backshore vegetation (m) on South Carolina sandy beaches (a = pristine sites, b = moderately impacted sites, c = highly impacted sites by only people, d = highly impacted sites by people and vehicles).

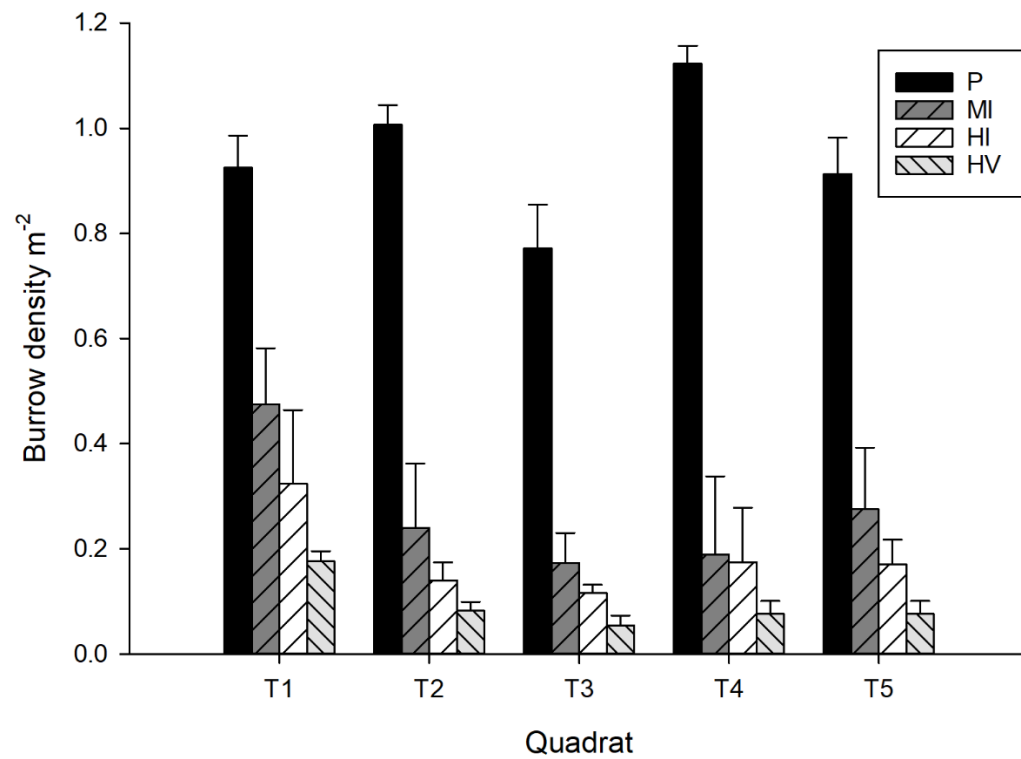


Figure 3.4. Ghost crab burrow density among impact types and height of the beaches. *O. quadrata* burrow density (burrows/m<sup>2</sup>  $\pm$  S.D.) on South Carolina sandy beaches with various disturbance levels and with height on the shore (transects).

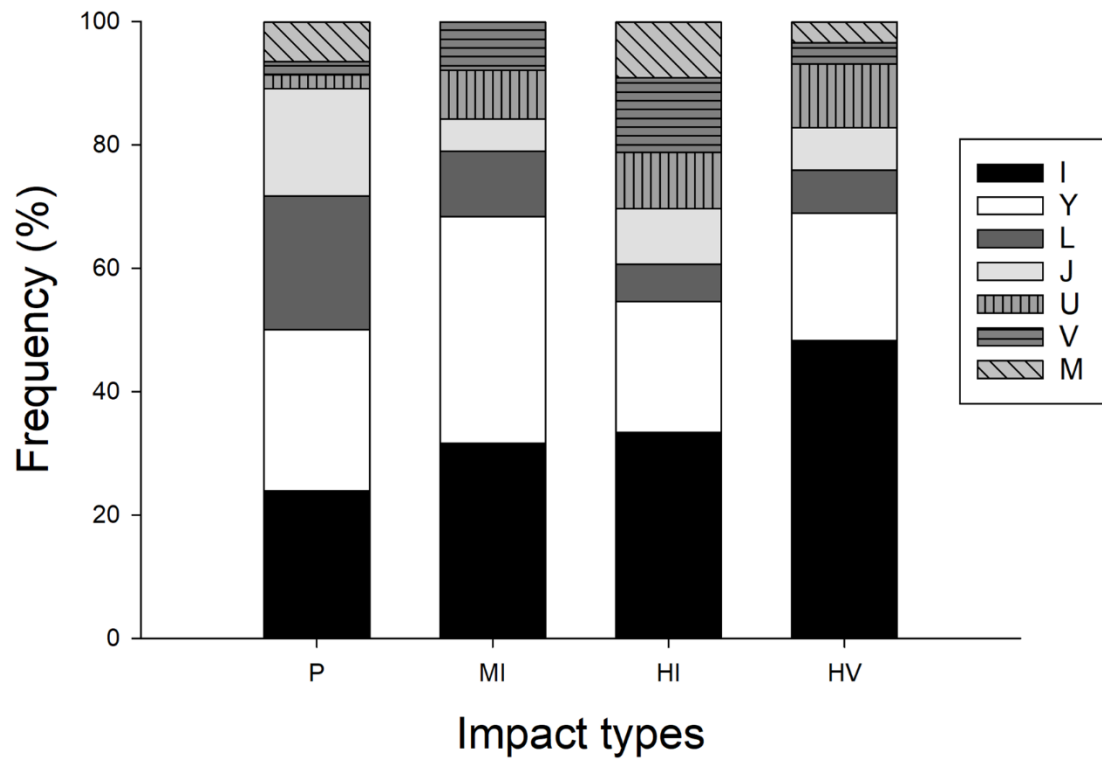


Figure 3.5. Variation in burrow shapes among impact types. Percent frequency (%) of *O. quadrata* burrow shapes among impact types on South Carolina beaches.



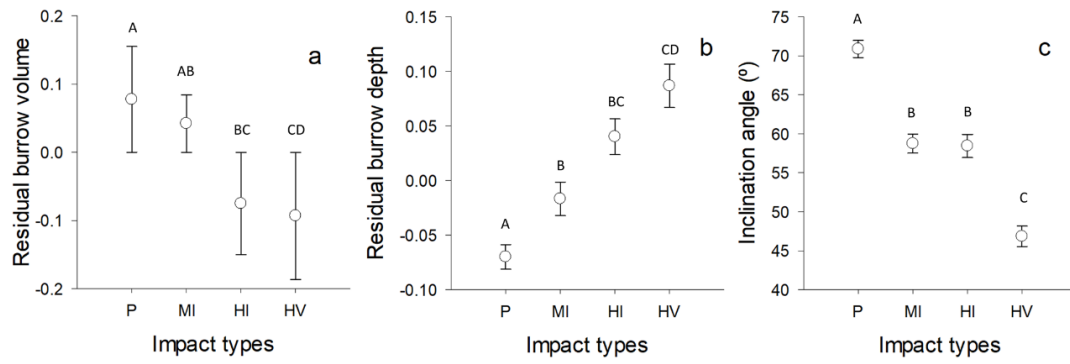


Figure 3.6. Burrow metrics among impact types. Variation in residual burrow volume (mean  $\pm$  S.E.) (a), residual burrow depth (mean  $\pm$  S.E.) (b) and inclination angle (mean  $\pm$  S.E.) for *O. quadrata* among impact types on South Carolina beaches. Capital letters within graph represent significant differences (c).

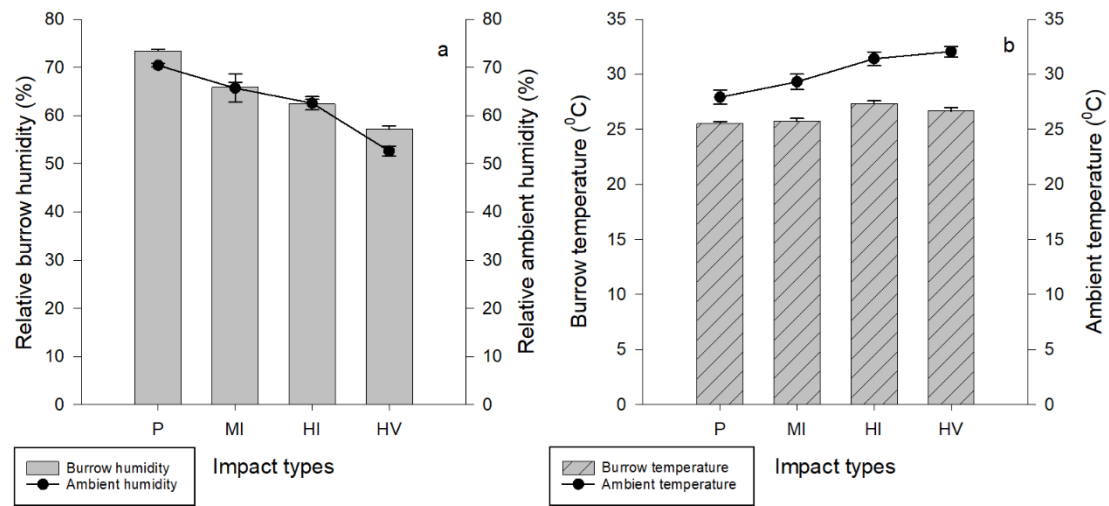


Figure 3.7. Environmental properties. Variation in environmental properties in *O. quadrata* burrows and among disturbance types. a) relative ambient and burrows humidity (mean  $\pm$  S.E.) among disturbance types, b) burrow and ambient temperature (mean  $\pm$  S.E.) among disturbance types.

### 3.11 Literature Cited

- Aheto, D., Asare, C., Mensah, E., & Aggrey-Fynn, J. (2011). Rapid assessment of anthropogenic impacts on exposed sandy beaches in Ghana using ghost crabs (*Ocypode* spp.) as ecological indicators. *MEJS.*, 3(2): 93–103.
- Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81(2), 169–193. <https://doi.org/10.1890/10-1510.1>
- Barros, F. (2001). Ghost crabs as a tool for rapid assessment of human impacts on exposed sandy beaches. *Biological Conservation*, 97(3): 399–404.  
[https://doi.org/10.1016/S0006-3207\(00\)00116-6](https://doi.org/10.1016/S0006-3207(00)00116-6)
- Bartoń, K. (2017). MuMIn: Multi-Model Inference. R package version 1.40.4.2017.
- Blickhan, R., & Full, R.J. (1987). Locomotion energetics of the ghost crab: II. Mechanics of the center of mass during walking and running. *Journal of Experimental Biology*, 130:155–174.
- Burnham, K.P., & Anderson, D.R. (2002). Model selection and inference: a practical information-theoretic approach. Springer-Verlag. 2002.
- Burrows, M., & Hoyle, G. (1973). The mechanism of rapid running in the ghost crab, *Ocypode ceratophthalma*. *Journal of Experimental Biology*. 1973; 58: 327–349.
- Cardoso, R.S, Barboza, C.A.M., Skinner, V.B., & Cabrini, T.M.B. (2016). Crustaceans as ecological indicators of metropolitan sandy beach health. *Ecological Indicators*, 62, 154–162. <https://doi.org/10.1016/j.ecolind.2015.11.039>

- Carignan, V., & Villard, M.A. (2002). Selecting indicator species to monitor ecological integrity: A review. *Environmental Monitoring and Assessment*, 78(1), 45–61.
- Chakrabarti, A. (1981). Burrow patterns of *Ocypode ceratophthalma* (Pallas) and their environmental significance. *Journal of Paleontology*, 55: 431–441.
- Chan, B.K.K., Chan, K.K.Y., & Leung, P.C.M. (2006). Burrow architecture of ghost crab *Ocypode ceratophthalma* on a sandy shore in Hong Kong. *Hydrobiologia*, 560(1), 43–49. <https://doi.org/10.1007/s10750-005-1088-2>
- Davenport, J., & Davenport, J.L. (2006). The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine, Coastal and Shelf Science*, 67(1–2), 280–292. <https://doi.org/10.1016/j.ecss.2005.11.026>
- Defeo, O., & de Alava, A. (1995). Effects of human activities on long-term trends in sandy beach populations: the wedge clam *Donax hanleyanus* in Uruguay. *Marine Ecology Progress Series*, 123: 72–82.
- Defeo, O., McLachlan, A., Schoeman, D.S., Schlacher, T.A., Dugan, J., Jones, A., Lastra, M., & Scapini, F. (2009). Threats to sand beach ecosystems: a review. *Estuarine, Coastal and Shelf Science*, 81(1), 1–12. <https://doi.org/10.1016/j.ecss.2008.09.022>
- Defeo, O., & Rueda, M. (2002). Spatial structure, sampling design and abundance estimates in sandy beach macroinfauna: some warnings and new perspectives. *Marine Biology*, 140(6): 1215–1225. <https://doi.org/10.1007/s00227-002-0783-z>

- de Oliveira, C.A.G., Souza, G.N., & Soares-Gomes, A. (2016). Measuring burrows as a feasible non-destructive method for studying the population dynamics of ghost crabs. *Marine Biodiversity*, 46:809–817. <https://doi.org/10.1007/s12526-015-0436-3>
- de Souza, G.N., Oliveira, C.A.G., & Tardem, A.S. (2017). Counting and measuring ghost crab burrows as a way to assess the environmental quality of beaches. *Ocean and Coastal Management*, 140: 1–10. <https://doi.org/10.1016/j.ocecoaman.2017.02.007>
- Dixon, R.W., Peters, S.L., & Townsend, C.G. (2015). Burrowing preferences of Atlantic ghost crab, *Ocypode quadrata*, in relation to sand compaction in Padre Island National Seashore, Texas. *Physical Geography*, 36(3): 188–201. <https://doi.org/10.1080/02723646.2015.1033182>
- Fisher, J., & Tevesz, M. (1979). Within-habitat spatial patterns of *Ocypode quadrata* (Fabricius) (Decapoda Brachyura). *Crustaceana*, 5:31–36.
- Folk, R.L. (1980). Petrology of sedimentary rocks. Hemphill Publishing Company.
- González, S.A., Yáñez-Navea, K., & Muñoz, M. (2014). Effect of coastal urbanization on sandy beach coleoptera *Phaleria maculate* (Kulzer, 1959) in northern Chile. *Marine Pollution Bulletin*, 83(1), 265–274. <https://doi.org/10.1016/j.marpolbul.2014.03.042>
- Gül, M.R., & Griffen, B.D. (2018). A reliable bioindicator of anthropogenic impact on the coast of South Carolina. *Southeastern Naturalist*, 17(2): 357–364.
- Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agros, C., .....Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, 319, 948–952. <https://doi.org/10.1126/science.1149345> PMID: 18276889

Hobbs, C.H., C.B. Landry, & Perry, J.E. (2008). Assessing anthropogenic and natural impacts on Ghost Crabs (*Ocypode quadrata*) at Cape Hatteras National Seashore, North Carolina. *Journal of Coastal Research*, 24(6), 1450–1458.

James, R.D. (2000). From beaches to beach environments: linking the ecology, human-use and management of beaches in Australia. *Ocean and Coastal Management*, 43, 495–514.

Jonah, F.E., Agbo, N.W., Agbet, W., Adjei-Boateng, D., & Shimba, M.J. (2015). The ecological effects of beach sand mining in Ghana using ghost crabs (*Ocypode* species) as biological indicators. *Ocean and Coastal Management*, 112: 18–24.

Kana, T.W. (1988). Beach erosion in South Carolina. South Carolina Sea Grant Consortium.

Lucrezi, S., Saayman, M., & van der Merwe, P. (2014). Impact of off-road vehicles (ORVs) on ghost crabs of sandy beaches with traffic restrictions: A case study of Sodwana Bay, South Africa. *Environmental Management*, 53, 520–533.  
<https://doi.org/10.1007/s00267-013-0223-5> PMID: 24370998

Lucrezi, S., Schlacher, T.A., & Robinson, W. (2009). Human disturbance as a cause of bias in ecological indicators for sandy beaches: experimental evidence for the effects of human trampling on ghost crabs (*Ocypode* spp.). *Ecological Indicators*, 9 (5): 913–921.  
<https://doi.org/10.1016/j.ecolind.2008.10.013>

Lucrezi, S., & Schlacher, T.A. (2010). Impacts of off-road vehicles (ORVs) on burrow architecture of ghost crabs (Genus *Ocypode*) on sandy beaches. *Environmental Management*, 45(6), 1352–1362. <https://doi.org/10.1007/s00267-010-9491-5>

Lucrezi, S., Schlacher, T.A., & Walker, S. (2009). Monitoring human impacts on sandy shore ecosystems: a test of ghost crabs (*Ocypode* spp.) as biological indicators on an urban beach. *Environmental Monitoring and Assessment.*, 152: 413–424.

<https://doi.org/10.1007/s10661-008-0326-2> PMID: 18563608

McLachlan, A., & Brown, A. (2006). *The ecology of sandy shores*. Elsevier.

Milne, L.J., & Milne, M.J. (1946). Notes on the behavior of the ghost crab. *American Naturalist*, 80:362–380.

Neves, F.M., & Bemvenuti, C.E. 2006. The ghost crab *Ocypode quadrata* (Fabricius, 1787) as a potential indicator of anthropogenic impact along the Rio Grande do Sul coast, Brasil. *Biological Conservation*, 133(4), 431–435.

Ojima, D.S., Galvin, K.A., & Turner, B.L. II. (1994). The global impact of land-use change. *BioScience*, 44(5), 300–304. <https://doi.org/10.2307/1312379>

Olds, S.J. Walker, J.X. Leon, J.M. Huijbers, M.A. Weston, A. Turra, G.A. Hyndes, R.A. Holt, & Schoeman, D.S. (2016a). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal, and Shelf Science*, 169, 56–73.

Packard, G.C., Boardman T.J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort?

*Comparative Biochemistry and Physiology Part A*, 122: 37–44.

[https://doi.org/10.1016/S1095-6433\(98\)10170-8](https://doi.org/10.1016/S1095-6433(98)10170-8)

Pombo, M., & Turra, A. (2013). Issues to be considered in counting burrows as a measure of Atlantic Ghost Crab populations, an important bioindicator of sandy beaches. PLoS ONE, 8(12):e83792.

Pombo, M., de Oliveira, A.L., Xavier, L.Y., Siegle, E., & Turra, A. (2017). Natural drivers of distribution of ghost crabs *Ocypode quadrata* and the implications of estimates from burrows. Marine Ecology Progress Series, 2017; 565: 131– 147.  
<https://doi.org/10.3354/meps11991>

R core team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rodrigues, E., Freitas, R., Delgado, N. de C., & Soares-Gomes, A. (2016). Distribution patterns of the ghost crab *Ocypode cursor* on sandy beaches of a tropical island in the Cabo Verde archipelago, Eastern Central Atlantic. African Journal of Marine Science, 38(2): 181–188. <https://doi.org/10.2989/1814232X.2016.1176602>

Sakai, K., & Türkay, M. (2016). Revision of the genus *Ocypode* with the description of a new genus, *Hoplocypode* (Crustacea: Decapoda: Brachyura). Memoirs of the Queensland Museum, 56(2), 665–793.

Sheppard, N., Pitt, K.A., & Schlacher, T.A. (2008). Sub-lethal effects of off-road vehicles (ORVs) on surf clams on sandy beaches. Journal of Experimental Marine Biology and Ecology, 380, 113–118. <https://doi.org/10.1016/j.jembe.2009.09.009>



Schlacher, T.A., Thompson, L., & Price, S. (2007). Vehicles versus conservation of invertebrates on sandy beaches: mortalities inflicted by off-road vehicles on ghost crabs.

Marine Ecology, 28(3), 354–367. <https://doi.org/10.1111/j.1439-0485.2007.00156.x>

Schlacher, T.A., Thompson, L.M.C., & Walker, S.J. (2008). Mortalities cause by off-road vehicles (ORVs) to a key member of sandy beach assemblages, the surf clam *Donax deltoids*. Hydrobiologia, 610, 345– 350. <https://doi.org/10.1007/s10750-008-9426-9>

Schlacher, T.A., S. Lucrezi, R.M. Connolly, C.H. Peterson, B.L. Gilby, B. Maslo, A.D.

Schlacher, T.A., & Lucrezi, S. (2010). Experimental evidence that vehicle traffic changes burrow architecture and reduces population density of ghost crabs on sandy beaches. Vie Milieu- Life Environ., 60: 313–320.

Schlacher, T.A., Lucrezi, S., Peterson, C.H., Connolly, R.D., Olds, A.D., Althaus, F., et al. (2016). Estimating animal populations and body sizes from burrows: Marine ecologists have their heads buried in the sand. Journal of Sea Research, 112: 55–64.

<http://dx.doi.org/10.1016/j.seares.2016.04.001>

Silva, W.T.A.F., & Calado, T.C.S. (2013). Number of ghost crab burrows does not correspond to population size. Central European Journal of Biology, 8(9): 843–847.

<https://doi.org/10.2478/s11535-013-0208-7>

Silva, W.T.A.F., & Calado, T.C.S. (2014). Burrow architectural types of Atlantic ghost crab, *Ocypode quadrata* (Fabricius, 1787) (Brachyura: Ocypodidae), in Brazil. bioRxiv.

<https://doi.org/10.1101/006098>

Solomon, M., Van Jaarsveld, A.S., Biggs, H.C., Knight, M.H. (2003). Conservation targets for viable species assemblages? *Biodiversity and Conservation*, 12: 2435–2441.

<https://doi.org/10.1023/A:1025805731366>

Steiner, A.J., & Leatherman, S.P. (1981). Recreational impacts on the distribution of ghost crab *Ocypode quadrata* Fab. *Biological Conservation*, 20, 111–122.

Suciu, M.C., Tavares, D.C., & Zalmon, I.R. (2018). Comparative evaluation of crustaceans as bioindicators of human impact on Brazilian sandy beaches. *Journal of Crustacean Biology*, ruy027. <https://doi.org/10.1093/jcbiol/ ruy027>

Trivedi, J.N., & Vachhrajani, K.D. (2016). On burrow morphology of the ghost crab *Ocypode ceratophthalmus* (Decapoda;Brachyura: Ocypodidae) from sandy shore of Gujarat, India. *International Journal of Marine Science.*, 6(15): 1–10.

<https://doi.org/10.5376/ijms.2016.06.0015>

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M. (1997) Human domination of Earth's ecosystems. *Science*, 277, 494–499.

<https://doi.org/10.1126/science.277.5325.494>

Wolcott, T.G. (1976). Uptake of soil capillary water by ghost crabs. *Nature*, 264: 756–757. <https://doi.org/ 10.1038/264756a0>

Wolcott, T.G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fabricius) on an ocean beach: scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31(1), 67–82.

Wolcott, T.G., & Wolcott, D.L. (1984). Impact of off-road vehicles on macroinvertebrates on a Mid-Atlantic beach. *Biological Conservation*, 29(3), 217–240.  
[https://doi.org/10.1016/0006-3207\(84\)90100-9](https://doi.org/10.1016/0006-3207(84)90100-9)

CHAPTER 4

COMBINED IMPACTS OF NATURAL AND ANTHROPOGENIC  
DISTURBANCES ON THE BIOINDICATOR *OCYPODE QUADRATA*  
(FABRICIUS, 1787)<sup>1</sup>

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<sup>1</sup>Gül MR. & Griffen BD. 2019. Combined impacts of natural and anthropogenic disturbances on the bioindicator *Ocypode quadrata* (Fabricius, 1787). *Journal of Experimental Marine Biology and Ecology*. 519: 151185  
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## 4.1 Abstract

Intense natural disturbances can dramatically affect populations in various ecosystems and existence of human influences can make the impacts of natural disturbance even greater by reducing the resilience of the populations. Here, we determine how the impacts of natural and anthropogenic disturbances alter population structure of a widespread bioindicator, the Atlantic ghost crab (*Ocypode quadrata*) by examining its burrow density, size and distribution before and after a hurricane on beaches with different levels of human impacts. We predicted that burrow density and size would show a dramatic decline after the hurricane mainly on the most human-impacted beaches. Because the smaller individuals of this species are highly sensitive to desiccation and thus normally occupy the lower parts of beaches, we further predicted that burrow distribution would not show any changes following the hurricane. We found that the density of ghost crab burrows strongly declined, but that this decline was less dramatic on beaches with higher levels of human impact. However, the decline in the burrow size 4- 7 days following hurricane disturbance indicates that the hurricane had greater impact on larger than on smaller individuals. Moreover, we found that the burrow distribution shifted after the hurricane. When the larger individuals were excluded from the system, the smaller individuals occupied the upper parts of the beach, suggesting that intra-specific interactions are the major mechanism determining the distribution pattern of ghost crabs on sandy shores, rather than physiological limitations (desiccation) that have previously been proposed. Our results suggest that, human influences make the populations less resilient against natural disturbances and that natural disturbances have a larger impact on larger individuals of this bioindicator species.

## 4.2 Introduction

Climate change has led to more frequent and intense high-energy weather events for the last 100 years (Emanuel 2005). Because of their large scale and destructive nature, natural extreme weather events can cause both short term (Burkholder et al. 2004, Widmer et al. 2004) and long term (Waide 1991) impacts on natural populations and communities. While scientists have long recognized the impacts of natural disturbances on populations, communities, and ecosystems, documenting these impacts can be challenging because it requires data from the system before the natural disturbance, which are often not available (Waide 1991, Widmer et al. 2004).

Natural disturbances can exert negative effects such as mass-mortality (Fourqurean & Rutten 2004, Milton et al. 1994), reduced diversity (Fourqurean & Rutten 2004, Willig & Camilo 1991), reduced density (Willig & Camilo 1991, Askins & Evert 1991), decreased individual sizes (Cannizzo & Griffen 2017, Hobbs et al. 2008), lower reproductive success (Roman et al. 1994, Woolbright 1991), and shifts in home ranges (Widmer et al. 2004). These impacts are seen in terrestrial (Pascarella 1998, Spiller et al. 1998, Waide 1991, Widmer et al. 2004), coastal (Cannizzo & Griffen 2017, Hobbs et al. 2008) and aquatic (Bailey & Secor 2016, Jurry et al. 1995, Mumby 1998, Roman et al. 1994, Willig & Camilo 1991) populations, communities and ecosystems. Species in these areas have evolved adaptations that enhance survival (Bailey & Secor 2016, Gunzburger et al. 2010, Swilling et al. 1998) and speed recovery (Burkholder et al. 2004). Yet, strong disturbances can neutralize these adaptations, leading to extensive recovery times.

Hurricanes are one type of natural catastrophe that can have detrimental impacts on coastal ecosystems. In addition, coastal ecosystems are under intense anthropogenic

pressure and human impacts have been increasing proportionally with increasing human population density in these areas for year (Davenport & Davenport 2006, Ojima et al. 1994). These anthropogenic impacts reduce the resiliency of coastal ecosystems and may therefore exacerbate the impacts of natural disturbances, such as hurricanes, in coastal areas (Mallin et al. 1999, 2002).

Sandy beaches are particularly exposed to both natural and anthropogenic disturbances. These highly erosional sites experience continual sediment fluxes (McLachlan & Brown 2006) that would appear to condition them for disturbance. Yet, sudden intense disturbances like hurricanes can result in mass mortality (Milton et al. 1994), decrease in the abundance of populations (Swilling et al. 1998, Hobbs et al. 2008, Witmer & Roelke 2014, Machado et al. 2019, Mathews & Maccarone 2019), as well as changes in biodiversity (Corte et al. 2017) and community structure (Machado et al. 2016). In addition to natural disturbances, sandy beaches are under intense human pressure (Davenport & Davenport 2006), which can increase the time to recovery for populations at these sites (Witmer & Roelke 2014, Machado et al. 2016, Mathews & Maccarone 2019).

Here we capitalized on an opportunity presented by a recent hurricane that passed over several of our previously-established study sites, to understand the extent to which human impacts make ghost crab populations less resilient to natural catastrophes. Hurricane Matthew reached category 5 strength in the Gulf of Mexico in 2016 before weakening along its path up the Florida and Georgia coasts and eventually reaching the South Carolina coast as a category 1 hurricane on October 8th, 2016 (Stewart 2017). The

storm moved northward, hugging the coast and creating strong winds, heavy rain, and storm surges along the South Carolina coast (Stewart 2017).

The Atlantic ghost crab, *Ocypode quadrata* is a semi-terrestrial species living on sandy shores from Rhode Island, USA to Brazil (Williams 1984). Atlantic ghost crabs respond to human disturbances by reducing their population densities and size distributions strongly, and individuals modify their burrowing behavior as human impacts intensify on sandy shores throughout this region (Gül & Griffen 2018a, 2018b). Thus, ghost crabs provide an opportunity for understanding the combined impacts of natural and human disturbances. At the time of the storm, we had an ongoing study that included several sites with different levels of human impact throughout the area impacted by the storm. We examined the combined impacts of human and hurricane disturbances by comparing changes in the density, size, and distribution of ghost crab burrows across individual beaches before and after the storm. We hypothesized that the density and size of burrows would decline more dramatically as a result of the hurricane in the sites that experienced greater human disturbance, but that the distribution pattern of burrows across the shore would not change due to the physiological limitations of the smaller individuals.

### **4.3 Materials and Methods**

To assess the impacts of Hurricane Matthew across levels of human disturbance on the Atlantic ghost crab, *O. quadrata* on South Carolina sandy beaches, a total of 15 sites under various levels of human disturbance were examined between 26th May and 17th October 2016 (Fig. 4.1). As a part of an ongoing study (Gül & Griffen 2018b), we collected data for 2,564 (1828 burrows before and 736 burrows after the hurricane) ghost



crab burrows across sites based on the variation in human impact levels. Thus, our pre-hurricane sampling occurred at various times from ~4 months to one week before the hurricane (September 28, 2016). We repeated the same procedures to measure ghost crab burrows at the same sites 4- 7 days after of the passing of the hurricane. We categorized our study sites based on the quantitative evaluations of the intensity of visitors, presence or absence of the mechanical cleaning machines and other vehicles on the sand.

According to this quantitative data, we grouped our study sites as moderately impacted sites (781 burrows before and 283 burrows after the hurricane), highly impacted sites by people (662 burrows before and 176 burrows after the hurricane) and highly impacted sites by people and vehicles (385 burrows before and 277 burrows after the hurricane). For a more detailed description of the study sites and their categorization, please see Gül and Griffen (2018a, 2018b).

We used the same sampling methods before and after the hurricane at each site. Specifically, we collected data for ghost crab burrow density, size and spatial distribution before and after Hurricane Matthew in rectangular quadrats (10m X 5m). We set up three quadrats parallel to the shore at each of five tidal heights starting from the backshore vegetation and moving seaward. Quadrats at the same tidal heights were spaced approximately 100 m from each other. We positioned the first sets of quadrats (T1) on the seaward part of the backshore (dune) vegetation. The fourth (T4) and the fifth (T5) sets of quadrats were set up just before and after the strandline. Because different sites had various beach widths, we positioned the second (T2) and third (T3) sets of quadrats between T1 and T4 spaced approximately equidistance from each other using visual observations. In some sites, dunes and vegetation were removed by the hurricane, so we

used the measurements of beach widths and quadrat placement before the hurricane to position our quadrats at similar tidal heights after the hurricane.

In each quadrat, we examined only the active ghost crab burrows. All measurements were made after sunset, when crabs generally leave their burrows (Wolcott 1978). We determined whether a burrow was active by observing the freshly disturbed sand and/or traces left by individuals. To determine the burrow density (burrows/m<sup>2</sup>), we counted the number of active burrows in each quadrat. If a burrow seemed to have more than one opening (determined when a smaller burrow opening without freshly dug sand was in very close proximity to a larger, active burrow opening), we examined only the main opening by observing the traces around it. To understand if Hurricane Matthew had an overall impact on the burrow density among various human impact types, we ran a generalized linear mixed effects model (GLMM) with a Poisson distribution. We treated the impact types, hurricane (pre or post) and the latitude (to control for differences in hurricane intensity as it moved northward along its path) as fixed factors. To control for any seasonal changes in burrow abundance because of the relatively long pre-hurricane sampling period, the sampling day (as Julian days) was treated as a random factor in this and all subsequent statistical analysis in this paper. We then applied a Tukey's HSD test to compare the burrow density among human impact types. Finally, we used separate generalized linear mixed effect models to compare the burrow density before and after the hurricane for each impact type.

We measured the diameter of the active ghost crab burrow opening (burrow size, hereafter) as the largest axis of the burrow mouth using a Vernier caliper (to the nearest 0.1mm) in each quadrat. This technique provides a highly accurate estimation of the

individual sizes of Atlantic ghost crabs ( $r^2 = 0.98$ ) (Wolcott 1978). To determine whether Hurricane Matthew had an impact on the ghost crab burrow size, we used a linear mixed effects model. Human impact type and hurricane (pre or post) were treated as fixed factors. Because it did not have an impact on the variation in burrow size (LME,  $t = 0.609$ ,  $p = 0.546$ ), we excluded latitude from our model for simplicity. We then applied a Tukey's HSD test to compare the burrow size at sites with different impact levels before and after the hurricane.

To compare the spatial distribution patterns of ghost crab burrows (density and size within quadrats T1- T5) within each impact type before and after the hurricane, we ran a generalized linear mixed effects models with a Poisson distribution for burrow density, and a general linear mixed effects model for the distribution of burrow size for each impact type, separately. We included the hurricane (pre or post) as a fixed factor and the quadrat (i.e. relative tidal height on the shore T1- T5) as an ordinal variable into all models.

#### **4.4. Results**

On South Carolina sandy shores, Hurricane Matthew had an overall negative impact on the ghost crab burrow density (GLMM,  $\text{estim.} = -95.03$ ,  $z = -2.211$ ,  $p = 0.027$ ) and this disturbance was more severe on the southern beaches where the hurricane was strongest (GLMM,  $\text{hurricane} \times \text{latitude}$ :  $\text{estim.} = 2.031$ ,  $z = -2.27$ ,  $p = 0.023$ ). This impact varied among sites based on human disturbance. The decline in the burrow density was significant in moderately impacted sites (27% decline, GLMM,  $\text{estim.} = -1.035$ ,  $z = -4.303$ ,  $p < 0.001$ ) and highly impacted sites by people (37% decline, GLMM,  $\text{estim.} = -1.492$ ,  $z = -5.589$ ,  $p < 0.001$ ), but not in the highly impacted sites by people and vehicles (13%

decline, GLMM, estim.=-0.266,  $z = -1.777$ ,  $p = 0.075$ ). A higher burrow density was still evident in moderately impacted sites compared to highly impacted sites by people (GLMM, estim.=0.0576,  $Z = 5.775$ ,  $p < 0.001$ ) and highly impacted sites by people and vehicles (GLMM, estim.=0.293,  $z = 3.157$ ,  $p = 0.016$ ) even after the hurricane. No difference was found in terms of burrow density after the hurricane between highly impacted sites (people vs. people and vehicles, GLMM, estim.= 0.273,  $z = -2.577$ ,  $p = 0.088$ ; Fig. 4.2a).

Hurricane Matthew led to a drastic decline in the ghost crab burrow size in the sites that experienced moderate impact (23% decline, LME, estim.=16.262,  $t = -11.758$ ,  $p < 0.001$ ), as well as heavy impact by people (24% decline, LME, estim.=15.031,  $t = -10.954$ ,  $p < 0.001$ ) and heavy impact by people and vehicles (19% decline, LME, estim.=10.132,  $t = -5.75$ ,  $p < 0.001$ ). However, while the mean burrow size was inversely proportional to the magnitude of the human impacts before the hurricane, it showed no difference in moderately impacted sites compared to highly impacted sites (by people: estim.=2.152, LME,  $t = 2.603$ ,  $p = 0.087$ ; by people and vehicles: LME, estim.=1.789,  $t = 0.928$ ,  $p = 0.933$ ) after the hurricane. Also, there was no difference in burrow size after the hurricane between sites that allowed vehicles on the sand and those that did not (LME, estim. =0.362,  $t = 0.186$ ,  $p = 0.988$ ; Fig. 4.2b).

The changes in burrow size and densities within beaches (from T1 to T5) before vs. after the hurricane varied between impact types. The burrow density across beaches declined significantly in sites highly impacted by people (GLMM, hurricane\*quadrat, estim.=-0.179,  $z = -2.861$ ,  $p = 0.0042$ ), but not in moderately impacted sites (GLMM, hurricane\*quadrat, estim.=-0.085,  $z = -0.021$ ,  $p = 0.983$ ) or in sites highly impacted by

people and vehicles (GLMM, hurricane\*quadrat, estim.=-0.001, z= -1.541, p = 0.123) after the hurricane (Fig. 4.3). The distribution of burrows in various sizes across beaches shifted after the hurricane in moderately impacted sites (LME, hurricane\*quadrat, estim.=-3.015, t= -6.315, p <0.001) and in highly impacted sites by people (LME, hurricane\*quadrat, estim.=-1.815, t= -3.978, p < 0.001) but not in highly impacted sites by people and vehicles (LME, hurricane\*quadrat, estim.=-0.33, t= -1.095, p = 0.274). However, smaller crabs mostly occupied the lower areas of the beach with larger crabs occupying the upper parts before the hurricane, the majority of the smaller burrows were observed at upper areas on the beach after the hurricane (Fig. 4.4).

#### **4.5 Discussion**

We have shown that both Hurricane Matthew and human disturbances had significant impacts on Atlantic ghost crab populations. Our results demonstrate that the hurricane caused a significant decline in the burrow density, particularly on shores that had less human impact. Additionally, our results show that the hurricane cause a decrease in burrow size regardless of the level of human disturbance. We have further shown that the hurricane had a disproportionately large impact on the abundance of large crabs, reducing the size distribution of crabs across the shore.

We found that the overall burrow density and size are each negatively impacted by both human and hurricane disturbance. Variations in burrow density and size across sites with various levels of human disturbance before Hurricane Matthew may result from vehicles driven on the sand (Wolcott & Wolcott 1984, Lucrezi & Schlacher 2010), individual burrowing characteristics (i.e. higher mortality is expected for individuals whose burrows are shallower than 25 cm, Schlacher et al. 2007), intense pedestrian

trampling (Neves & Bemvenuti 2006, Lucrezi et al. 2009), lower life expectancy of the individuals in sites under human disturbance (de Souza et al. 2017), and direct handling by people (Gül & Griffen 2018b).

After the hurricane, both burrow density and size declined in all our study sites. The decrease in burrow in burrow density may reflect direct mortality of crabs that occurred during the storm (Hobbs et al. 2008). We found that before the storm, larger individuals mostly occurred in the upper zones of the beach and that smaller individuals mostly burrow lower down, next to the strand line and in the swash zone. The shift in size distribution after the storm to smaller crabs than were found before the storm may have multiple possible explanations. First, all crabs may have shifted to higher ground during the storm, moving large crabs that were previously on the upper shore into coastal uplands and out of our sampling area, and moving smaller crabs from lower shore regions to higher up on the shore. We believe that this explanation is unlikely to fully explain the observed pattern. Many of our sites that are highly impacted by people occurred in Myrtle Beach, an area where roads, shops, hotels, and other hard surfaces exist immediately adjacent to the beach and extend far inland, eliminating possible inland burrowing locations. Any crabs that moved up into this area to avoid the storm surge would have been likely to return to the shore before our sampling that occurred 4-7 days post-hurricane. Second, loss of predominantly large crabs may reflect differential mortality due to differences across the shore in sediment compaction and associated burrow stability (Scott et al. 2016). Small crabs that burrow lower on the shore may have been protected to a greater extent during the hurricane than larger crabs that burrow in the less compacted sand on the upper shore (areas lower on the shore can also experience

lower wave action during a storm because they are in deeper water, Spencer et al, 2015). In this scenario, the greater occurrence of small crabs higher on the shore post-hurricane may reflect the movement of these crabs to higher elevations post-hurricane after the removal of competitively superior larger crabs.

In addition to the reduction in large crabs on the shore post-hurricane, we also documented the appearance of numerous very small individuals (burrow size <10 mm). These individuals were completely absent during our pre-hurricane sampling, and most certainly reflect new recruits to our study sites. Given the short time interval between the hurricane and our post-hurricane sampling (4-7 days), it is unlikely that the bulk of these recruits occurred after the hurricane. Instead, they likely recruited after our pre-hurricane sampling (i.e., up to 4 months before the hurricane, depending on the site), but before the hurricane arrived.

The impacts of Hurricane Matthew reported here are consistent with a previous study by Hobbs et al. (2008) conducted on North Carolina sandy shores. They observed a dramatic decline in ghost crab burrow size after three high-energy weather events due to mortality of large individuals, and this mortality was higher on beaches where off-road vehicles were allowed than on more protected shores where they were not allowed. Further, they observed an overall increase in the total density of crab burrows in the two months following the storms, though these burrows were of predominantly small sizes. Consistent with the second hypothesis outlined above, these patterns suggest a loss of large crabs due to disproportionate mortality during the storm events in their study area, followed by an increase in recruitment of juvenile crabs to these same shores, which as they suggest, was due to a lack of competition from larger crabs.

Atlantic ghost crabs can move to the upper parts of the beaches (e.g. dunes) and to inland areas on the shores (Leber 1982, Lucrezi & Schlacher 2014). Some individuals may respond to intense coastal storms by moving into the dunes and inland parts of the shores, returning to the sand after the water and threat has subsided. However, as stated above, in many of our study sites, buildings start immediately next to the sand and there are no real dunes. So, individuals did not have the opportunity to move inland and develop burrows on higher ground in response to the hurricane, possibly leading to higher mortality in our sites. If this hypothesis is true, it suggests that the impacts of humans are not just through the number of people that visit the beaches and the ability to drive vehicles on the beach, but also through the construction of buildings next to the beach. In this case, storms interact with hard structures (buildings, roads, beach walls) to impact ghost crabs. This hypothesis is supported by a study on the sandy shores of Queensland, Australia, where Lucrezi et al. (2010) reported a lower ghost crab burrow density at sites with seawalls compared to unarmored sites after the passage of a relatively small storm.

Our results have revealed that ghost crabs have different distribution patterns before and after a hurricane disturbance. We found that larger individuals generally prefer upper parts of the beach, with smaller crabs excavate their burrows close to the strand line. Additionally, burrow densities were highest at the upper and lower edges of the beaches. Even though ghost crabs, as semi-terrestrial organisms, can excavate burrows in both dry and wet areas (Fisher and Tevesz 1979), they frequently need to moisten their gills for respiration, and smaller individuals moisten their gills more frequently (Wolcott 1976). An inverse relationship between human impacts and the moisture content of the sands has previously been reported (Gül & Griffen 2018b,



Lucrezi & Schlacher 2010, Steiner & Leatherman 1981), explaining the general pattern of smaller burrows lower down on the shore. However, other explanations have been used to explain this distribution pattern, including territoriality and cannibalism by larger individuals (Chakrabarti 1981, Gül & Griffen 2018b, Fisher & Tevesz 1979). Here, we have demonstrated that the smaller individuals occupy the upper portions of the beach after the hurricane and that burrows are distributed more evenly on the shore when larger individuals were removed by the hurricane. Based on this observation, we conclude that intra-specific interactions (e.g. territoriality and/or cannibalism) are the major mechanisms that explains the variation in the distribution pattern between impact types on sandy shores.

In conclusion, we have shown that human disturbance and natural disturbance can both act to alter the abundance and size distribution of this bioindicator species. The density of human populations in coastal areas, and their use of the shore, is likely to increase in the future. Similarly, the intensity of storms and their impacts on coastal areas are also expected to increase moving forward because of climate change (Emanuel, 2005). These increasing stressors are likely to interact to negatively impact coastal species in unexpected ways. Understanding these combined impacts on natural populations is crucial for forecasting the possible responses and adaptations of those populations to future disturbances, and for effectively managing coastal areas. Hence, our results suggest that natural disturbances may cause detrimental impacts on populations in terms of their density and individual sizes and human influences may exacerbate those impacts by lowering the resilience of the populations.

#### **4.6 Acknowledgments**

We wish to thank Esra Erdil Gül for her assistance in field observations. Funding was provided by the Ministry of National Education, Republic of Turkey (to MRG).

#### 4.7 Figures

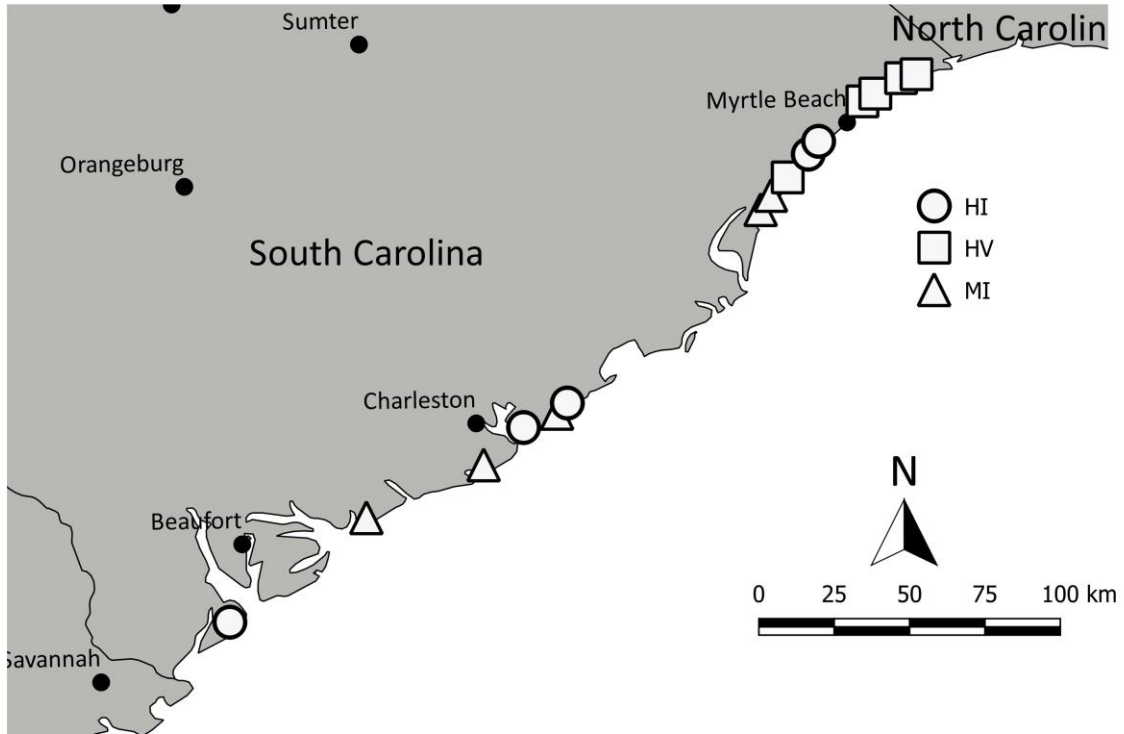


Figure 4.1. Map of the study sites on South Carolina coast. MI represents the moderately impacted sites, HI represents the highly impacted sites by people and HV represents highly impacted sites by people and vehicles. Map was created with QGIS v. 2.18.24 software.

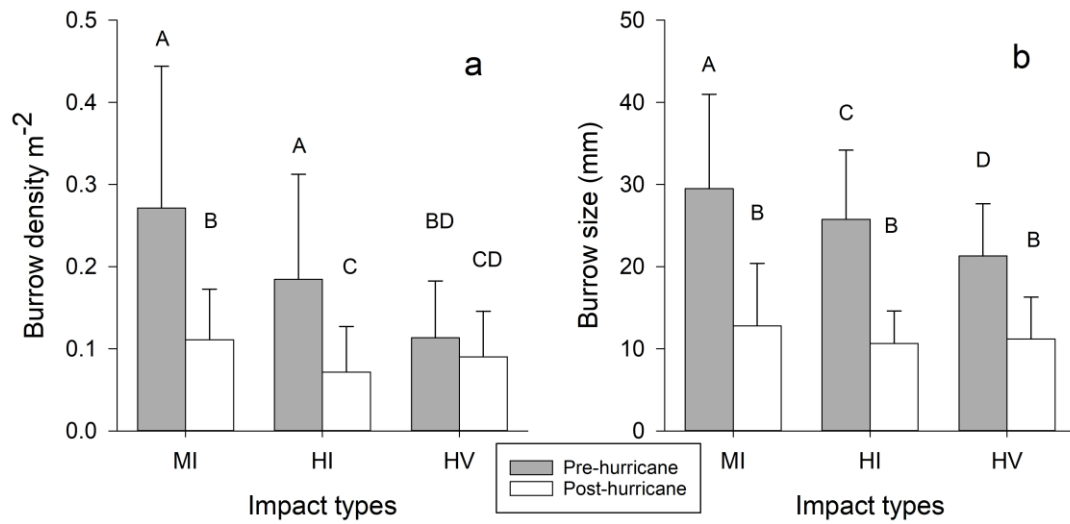


Figure 4.2. (a) Mean burrow density ( $\pm$ S.D.) of *O. quadrata* obtained before and after the hurricane among impact levels. (b) Mean burrow opening diameter ( $\pm$ S.D.) of *O. quadrata* measured before and after the hurricane among impact levels. Letters above the bars represent the results of Tukey's HSD test. MI represents the moderately impacted sites ( $n = 781$  before and 283 after the hurricane), HI represents the highly impacted sites by people ( $n = 662$  before and 176 after the hurricane) and HV represents highly impacted sites by people and vehicles ( $n = 385$  before and 277 after the hurricane).

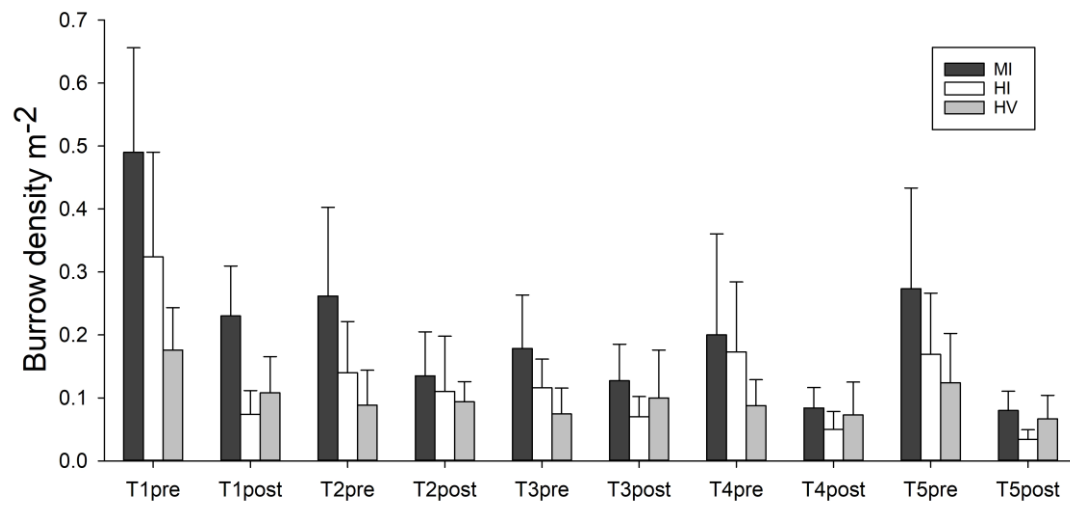


Figure 4.3. Mean burrow density ( $\pm$ S.D.) of *O. quadrata* on South Carolina sandy shores with tidal height on the shore and with various human impact levels before and after Hurricane Matthew.

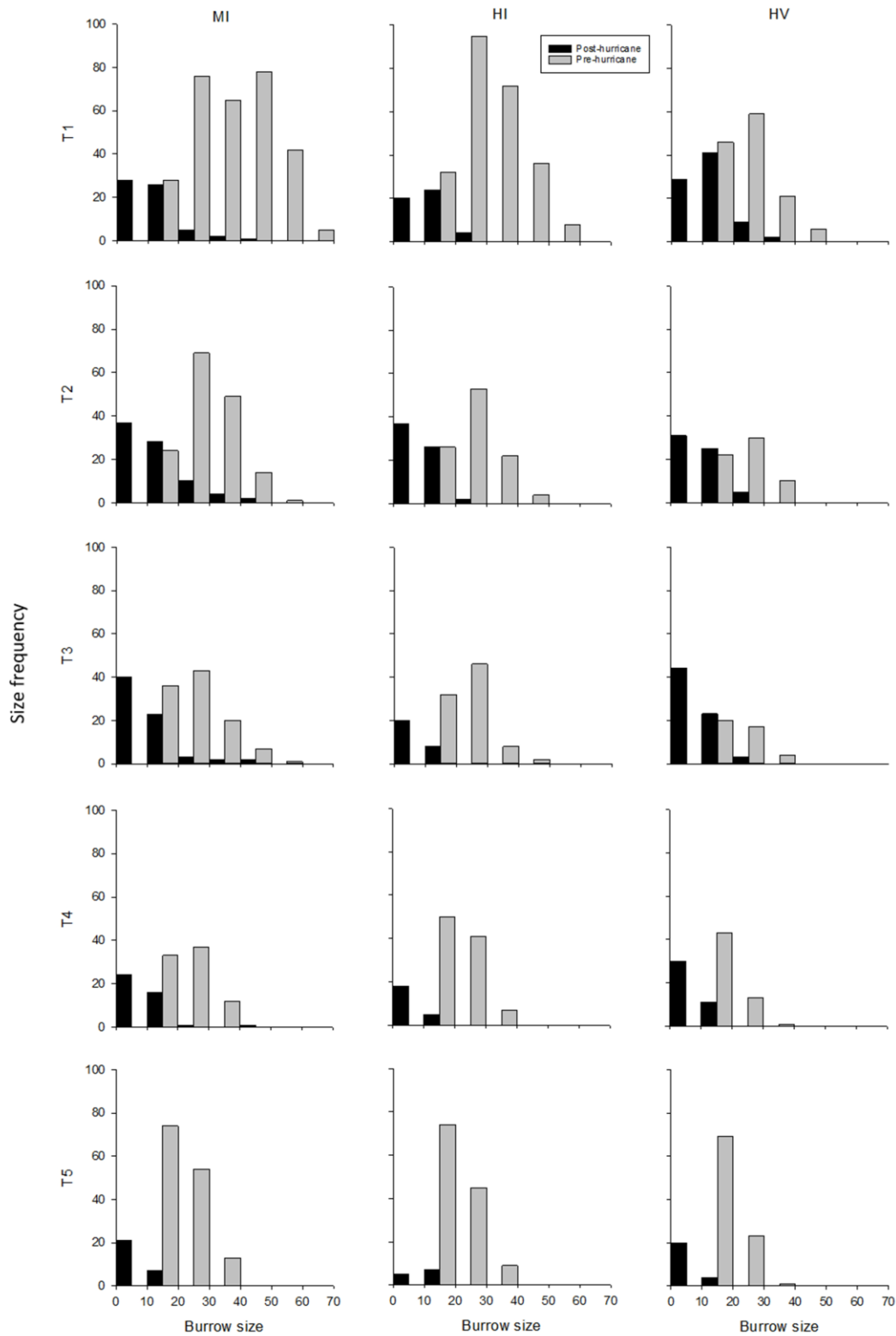


Figure 4.4. Size frequency variation of the burrow opening of *O. quadrata* measured at different tidal height among human impact levels before and after Hurricane Matthew.

#### 4.8 Literature Cited

- Askins, R.A., Ewert, D.N., 1991. Impact of Hurricane Hugo on Bird Populations on St. John, U.S. Virgin Islands. *Biotropica*. 23(4a), 481- 487.
- Bailey, H., Secor, D.H., 2016. Coastal evacuations by fish during extreme weather events. *Sci. Rep.* 6, 30280. doi: 10.1038/srep30280.
- Burkholder, J., Eggleston, D., Glasgow, H., Brownie, C., Reed, R., Janowitz, G., Posey, M., Melia, G., Kinder, C., Corbett, R., Toms, D., Alphin, T., Deamer, N., Springer, J., 2004. Comparative impacts of two major hurricane seasons on the Neuse River and western Pamlico Sound ecosystems. *PNAS*. 101(25), 9291- 9296.
- Cannizzo, Z.J., Griffen, B.D., 2017. Habitat-specific impacts of Hurricane Matthew on a range-expanding species. *Hydrobiologia*. 809(1), 79- 89. <https://doi.org/10.1007/s10750-017-3449-z>.
- Chakrabarti, A., 1981. Burrow patterns of *Ocypode ceratophthalma* (Pallas) and their environmental significance. *J. Palaeontol.* 55, 431- 441.
- Corte, G.N., Schlacher, T.A., Checon, H.H., Barboza, C.A.M., Siegle, E., Coleman, R.A., Amaral, A.C.Z., 2017. Storm effects on intertidal invertebrates: increased beta diversity of few individuals and species. *PeerJ*. 5:e3360. <https://doi.org/10.7717/peerj.3360>
- Davenport, J., Davenport, J.L., 2006. The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine Coastal and Shelf Science*, 67(1-2), 280- 292.
- Emanuel, K., 2005. Increasing destructiveness of tropical cyclones over the past 30 years. *Nature*. 436, 686- 688.

- de Souza, G.N., Oliveira, C.A.G., Tardem, A.S. Counting and measuring ghost crab burrows as a way to assess the environmental quality of beaches. *Ocean and Coastal Management*, 140, 1- 10. <https://doi.org/10.1016/j.ocecoaman.2017.02.007>.
- Fisher. J., Tevesz, M., 1979. Within-habitat spatial patterns of *Ocypode qudrata* (Fabricius) (Decapoda Brachyura). *Crustaceana*. 5, 31- 36.
- Fourqurean, J.W., Rutten, L.M., 2004. The Impact of Hurricane Georges on Soft-Bottom, Back Reef Communities: Site- and Species- Specific Effects in South Florida Seagrass Beds. *Bulletin of Marine Science*, 75(2), 239- 257.
- Gunzburger, M.S., Hughes, W.B., Barichivich, W.J., Staiger, J.S., 2010. Hurricane storm surge and amphibian communities on coastal wetlands of northwestern Florida. *Wetlands Ecology and Management*, 18, 651- 663. <https://doi.org/10.1007/s11273-010-9185-z>.
- Gül, M.R., Griffen, B.D., 2018a. A Reliable Bioindicator of Anthropogenic Impact on the Coast of South Carolina. *Southeastern Naturalist*, 17(2), 357- 364. <https://doi.org/10.1656/058.017.0217>.
- Gül, M.R., Griffen, B.D., 2018b. Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores. *PLoS ONE*. 13(12), e0209977. <https://doi.org/10.1371/journal.pone.0209977>
- Hobbs, III, C.H., Landry, C.B., Perry, III, J.E., 2008. Assessing Anthropogenic and Natural Impacts on Ghost Crabs (*Ocypode quadrata*) at Cape Hatteras National Seashore, North Carolina. *Journal of Coastal Research*, 24(6), 1450- 1458.
- Jury, S.H., Howell, W.H., Watson, III, W.H., 1995. Lobster movements in response to a hurricane. *Marine Ecology Progress Series*, 199, 305- 310.



Leber, K.M., 1982. Seasonality of macroinvertebrates on a temperate, high wave energy sandy beach. *Bulletin of Marine Science*, 32, 86- 98.

Lucrezi, S., Schlacher, T.A., 2010. Impacts of off-road vehicles (ORVs) on burrow architecture of ghost crabs (Genus *Ocypode*) on sandy beaches. *Environmental Management*, 45(6), 1352- 1362. <https://doi.org/10.1007/s00267-010-9491-5>.

Lucrezi, S., Schlacher, T.A., 2014. The ecology of ghost crabs – a review. *Oceanography and Marine Biology: An annual Review*, 52, 201- 256.

Lucrezi, S., Schlacher, T.A., Robinson, W., 2010. Can storms and shore armouring exert additive effects on sandy-beach habitats and biota? *Marine and Freshwater Research*, 61, 951- 962. <https://doi.org/10.1071/MF09259>.

Lucrezi, S., Schlacher, T.A., Robinson, W., 2009. Human disturbance as a cause of bias in ecological indicators for sandy beaches: experimental evidence for the effects of human trampling on ghost crabs (*Ocypode* spp.). *Ecological Indicators* 9(5), 913- 921. <https://doi.org/10.1016/j.ecolind.2008.10.013>.

Machado, P., Costa, L.L., Suciú, M.C., Tavares, D.C., Zalmon, I.R., 2016. Extreme storm wave influence on sandy beach macrofauna with distinct human pressures. *Marine Pollution Bulletin*, 107, 125- 135. <http://dx.doi.org/10.1016/j.marpolbul.2016.04.009>

Machado, P., Tavares, D.C., Zalmon, I.R., 2019. Synergistic effect of extreme climatic events and urbanization on population density of the ghost crab *Ocypode quadrata* (Fabricius, 1787). *Marine Ecology*, 40:e12525. <https://doi.org/10.1111/maec.12525>

Mallin, M.A., Posey, M.H., McIver, M.R., Parsons, D.C., Ensign, S.H. Alphin, T.D., 2002. Impacts and Recovery from Multiple Hurricanes in a Piedmont-Coastal Plain River System. *BioScience*. 52(11), 999- 1010.

Mallin, M.A., Posen, M.H., Shank, G.C., McIver, M.R., Ensign, S.H., Alphin, T.D., 1999. Hurricane effects on water quality and benthos in the Cape Fear Watershed: Natural and anthropogenic impacts. *Ecological Applications*, 9(1), 350, 362.

Mathews, P.L., Maccarone, A.D., 2019. Responses by Atlantic Ghost Crab (*Ocypode quadrata*) Populations to Hurricane Impacts on a Texas Beach: A 10-Year Study, *Transactions of the Kansas Academy of Science*. 122(1-2), 59-68.  
<https://doi.org/10.1660/062.122.0106>

McLachlan, A., Brown, A., 2006. The ecology of sandy shores. Elsevier.

Milton, S.L., Leone-Kabler, S., Schulman, A.A., Lutz, P.L., 1994. Effects of Hurricane Andrew on the Sea Turtle Nesting Beaches of South Florida. *Bulletin of Marine Science*, 54(3), 974- 981.

Neves, F.M., Bemvenuti, C.E., 2006. The ghost crab *Ocypode quadrata* (Fabricius, 1787) as a potential indicator of anthropogenic impact along the Rio Grande do Sul coast, Brazil. *Ecological Indicators*, 133(4), 431- 435.  
<https://doi.org/10.1016/j.biocon.2006.04.041>.

Ojima, D.S., Galvin, K.A., Turner II, B.L., 1994. The global impact of land-use change. *BioScience*. 44(5), 300- 304. <https://doi.org/10.2307/1312379>.

Pascarella, J.B., 1998. Hurricane Disturbance, Plant-Animal Interactions, and the Reproductive Success of a Tropical Shrub. *Biotropica*. 30(3), 416- 424.

Roman, C.T., Aumen, N.G., Trexler, J.C., Fennema, R.J., Loftur, W.F., Soukup, M.A., 1994. Hurricane Andrew's Impact on Freshwater Resources. *BioScience*. 44(4), 247- 255.

Schlacher, T.A., Thompson, L., Price, S., 2007. Vehicles versus conservation of invertebrates on sandy beaches: mortalities inflicted by off-road vehicles on ghost crabs. *Marine Ecology*, 28(3), 354- 367. <https://doi.org/10.1111/j.1439-0485.2007.00156.x>.

Scott, T., Masselink, G., O'Hare, T., Saulter, A., Poate, T., Russell, P., Davidson, M., Conley, D., 2016. The extreme 2013/2014 winter storms: Beach recovery along the southwest coast of England. *Marine Geology*, 382, 224- 241. <https://doi.org/10.1016/j.margeo.2016.10.011>.

Spencer, T., Brooks, S.M., Evans, B.R., Tempest, J.A., Möller, I., 2015. Southern North Sea storm surge event of 5 December 2013: Water levels, waves and coastal impacts. *Earth- Science Reviews*, 146, 120- 145. <https://doi.org/10.1016/j.earscirev.2015.04.002>.

Spiller, D.A., Losos, J.B., Schoener, T. W., 1998. Impact of a Catastrophic Hurricane on Island Populations. *Science*. 281, 695- 697.

Steiner, A.J., Leatherman, S.P., 1981. Recreational impacts on the distribution of ghost crab *Ocypode quadrata*. *Biological Conservation*, 20, 111- 122.

Stewart, S.R., 2017. Tropical Cyclone Report: Hurricane Matthew. National Hurricane Center. 1- 96.

Swilling, W.R., Wooten, M.C., Holler, N.R., Lynn, W.J., 1998. Population Dynamics of Alabama Beach Mice (*Peromyscus polionotus ammobate*) following Hurricane Opal. *American Midland Naturalist*, 140, 287- 298.

Waide, R.B., 1991. Summary of the Response of Animal Populations to Hurricanes in the Caribbean. *Biotropica*. 23(4a), 508- 512.

- Widmer, O., Saïd, S., Miroir, J., Duncan, P., Gaillard, J.M., Klein, F., 2004. The effects of hurricane Lothar on habitat use of roe deer. *Forest Ecology and Management*, 195, 237- 242.
- Williams, A.B., 1984. Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press, Washington D.C.
- Willig, M.R., Camilo, G.R., 1991. The Effect of Hurricane Hugo on Six Invertebrate Species in the Luquillo Experimental Forest of Puerto Rico. *Biotropica*. 23(4a), 455- 461.
- Witmer, A.D., Roelke, D.L., 2014. Human interference prevents recovery of infaunal beach communities from hurricane disturbance. *Ocean and Coastal Management*, 87, 52-60. <http://dx.doi.org/10.1016/j.ocecoaman.2013.11.005>
- Wolcott, T.G., 1976. Uptake of soil capillary water by ghost crabs. *Nature*. 264, 756–757.
- Wolcott, T.G., 1978. Ecological role of ghost crabs, *Ocypode quadrata* (Fabricius) on an ocean beach: scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31(1), 67- 82.
- Wolcott, T.G., Wolcott, D.L., 1984. Impact of off-road vehicles on macroinvertebrates on a Mid-Atlantic beach. *Biological Conservation*, 29(3), 217- 240. [https://doi.org/10.1016/0006-3207\(84\)90100-9](https://doi.org/10.1016/0006-3207(84)90100-9).
- Woolbright, L.L., 1991. The Impact of Hurricane Hugo on Forest Frogs in Puerto Rico. *Biotropica*. 23(4a), 462- 467.

## CHAPTER 5

### CHANGES IN CLAW MORPHOLOGY OF A BIOINDICATOR SPECIES ACROSS HABITATS THAT DIFFER IN HUMAN DISTURBANCE<sup>1</sup>

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<sup>1</sup>Gül MR. & Griffen BD. Changes in claw morphology of a bioindicator species across habitats that differ in human disturbance. Submitted to *Hydrobiologia*.

## 5.1 Abstract

Variations in the morphological characteristics are frequently important determinants of the physiology, ecology and inter and intra-specific interactions of organisms. Various causative drivers have been investigated for this morphological variation, such as sexual selection, variations in the ability to exploit available resources, habitat characteristics, and other environmental factors. However, it is not clear whether human disturbance has any selective role in creating morphological variations other than simply reducing the body size of individuals. We used a widespread bioindicator species, the Atlantic ghost crab *Ocypode quadrata*, to measure how human disturbance influences morphological claw variation. We examined the length, height and depth of major and minor claws of crabs at 12 South Carolina sandy beaches with various levels of human disturbance. Crabs from disturbed sites appeared to have smaller claws compared to crabs from pristine sites. Additionally, the claw sizes varied between males and females with relation to the human disturbance levels. Female crabs had lower relative claw volume compared their male conspecifics at disturbed sites. Our results suggest that the magnitude of the human disturbance experienced by populations may have a selective role on the morphology of individual organisms that can differ between males and females. These morphological changes likely have ecological and physiological consequences.

## 5.2. Introduction

Morphological variation plays a key role in the ecology, survival and the physiology of organisms. Numerous studies show that morphological differences lead to

differential exploitation of resources (Yamada & Boulding 1998, Buck et al. 2003, Jaroensutasinee & Jaroensutasinee 2015), potentially contributing to the physiological condition of organisms. Additionally, morphological size differences can determine social structures and influence the outcome of intra- and interspecific interactions (e.g. agonistic behaviors, predator-prey interactions) (Sneddon et al. 2000, Hernáez & João 2018, Cannizzo et al. 2019, Rico-Guevara & Hurme 2019).

Morphological variation between males and females is a widespread phenomenon in nature (reviewed in Shine 1989, Anderson 1994, Rico-Guevara & Hurme 2019), various hypotheses have been proposed to explain mechanisms behind this morphological shift. An early and ongoing explanation is sexual selection (Shine 1989, Claverie & Smith 2010, Cothran & Jeyasingh 2010, Rico-Guevara & Hurme 2019). Sexual selection can cause morphological divergence between males and females if one sex preferentially selects larger mates of the other gender in order to optimize reproductive success (Shine 1989, Anderson 1994, Maklakov et al. 2004). Additionally, sexual selection may lead to morphological divergence due to competition between members of the same sex for access to mates (Serrano-Meneses et al. 2007, Neff & Svensson 2013, Clutton- Brock 2017). Morphological divergence between genders may also arise from resource availability if the genders differ in their ability to exploit resources (Shine 1989, Smith & Palmer 1994, Cothran & Jeyasingh 2010, Nolen et al. 2017). Finally, habitat characteristics may have different selective impacts on each gender (Shine 1989, Giri & Loy 2008, Cannizzo et al. 2019).

Environmental factors may also lead to morphological variation across sites irrespective of gender. For example, crabs living in Argentinian lakes have shorter but

wider rostrum compared to conspecifics living in rivers (Giri & Loy 2008). Also, mangrove tree crabs living in salt marshes have larger claws compared to conspecifics living in dock and mangrove habitats (Cannizzo et al. 2019). Given the occurrence of morphological variation across habitat types, we may expect that extensive habitat modifications by humans that lead to large differences in habitat quality within a single type of habitat may also have implications for the morphology of animals.

Coastal regions experience strong human influence due to their ecosystem services (Davenport & Davenport 2006, Halpern et al. 2008, Barbier et al. 2011), and this influence is likely to become stronger in the future as the size of human coastal populations continue to increase (Vitousek et al. 1997, Davenport & Davenport 2006, Halpern et al. 2008). Species living in coastal regions already show signs of stress (Vitousek et al. 1997, Halpern et al. 2008, Defeo et al. 2009), with populations responding to human influence by reducing individual organismal sizes and population densities (Neves & Bemvenuti 2006, Hobbs et al. 2008, Cardoso et al. 2016, Schlacher et al. 2016, Gül & Griffen 2018a, 2018b, Suciú et al. 2018). Various mechanisms have been proposed to explain smaller individual sizes under human disturbance. Vehicles on the beach lead to direct mortalities in crabs by crushing (Wolcott & Wolcott, 1984; Schlacher et al. 2007). Larger crabs may experience a higher mortality compared to the smaller crabs if they emerge their burrows more often (Wolcott & Wolcott, 1984). In addition to the mortalities by vehicles, direct handling by people at disturbed sites can be an important determinant of the smaller body sizes of the crabs, since people mostly catch the larger ones (Gül & Griffen 2018b). Moreover, reduced food availability and/or quality is likely to cause a lower growth rates in ghost crabs at disturbed sites (Stelling-



Wood et al. 2016, Gül & Griffen in review (a)). Variations in energetic balance due to changes in behaviors and daily activities in ghost crabs between various disturbance levels, also, may explain the differences in the body size of crabs between heavily disturbed and moderately disturbed or pristine sites (Gül & Griffen (in review(b))). Yet the impacts of human disturbance on size-independent morphological features has received less attention.

The Atlantic ghost crab *Ocypode quadrata*, a semi-terrestrial organism, lives on sandy shores (Milne & Milne 1946), and is widely used as a bioindicator of human impacts because of its strong responses to different types of impacts caused by human (Wolcott & Wolcott 1984 Neves & Bemvenuti 2006, Gül & Griffen 2018a, 2018b, Suciu et al. 2018) and natural disturbances (Hobbs et al. 2008, Gül & Griffen 2019). As with other species, ghost crabs also display smaller individual size and lower population abundance on disturbed shores (Shclacher et al. 2016; Gül & Griffen 2018a, 2018b). In addition, ghost crabs also alter their burrowing behavior and burrowing energetic demand under human influence (Gül & Griffen in review (b)). Moreover, this species forms an important trophic link as a consumer of small beach organisms (clams, mole crabs) and a prey of higher trophic levels (Wolcott, 1978; Fisher & Tevesz, 1979). Additionally, their easily recognizable size and special behaviors (e.g. burrowing and foraging out on the open shore) make them advantageous organisms to work with (Neves & Bemvenuti 2006, Pombo & Turra 2013).

The impacts of human disturbances on natural populations have been widely investigated by observing the population densities and individual sizes of bioindicator species (Carignan & Villard 2002, Spellerberg 2005, Cortes et al. 2013). However, it is

not clear whether human disturbance plays a selective role in eliciting morphological differences other than the reductions in body size that have been reported. If human impacts can be shown to correlate with other forms of morphological change, this may further assist in forecasting the future conditions of disturbed populations as human use of coastal areas continues to rise. Therefore, we used the Atlantic ghost crab as a model organism to understand whether human disturbance can be correlated with variation in the claw morphology of natural populations. Changes in burrowing behaviors (i.e. burrow fidelity and longevity) in ghost crab behavior under different levels of human disturbance leads to energetic impacts that differ across disturbance levels (Gül & Griffen in review(b)), suggesting a potential shortage of available energy sources. Additionally, there are possible shortages in the amount of available energy for the crabs at disturbed sites because human disturbance leads to a dramatic decline in the common prey items of the ghost crabs (González et al. 2014, Cardoso et al. 2016, Frota et al. 2019, Laitano et al. 2019). Thus, we predicted that female ghost crabs would have smaller claw size at the sites with high human disturbance compared to conspecifics from pristine and moderately impacted sites due to a potential trade-off between energy allocation to reproduction vs. growth. We also predicted that males would have smaller claw size with human disturbance, but that these changes would be less dramatic compared to females due to a faster claw growth compared to females (Haley 1969).

## 5.3 Materials and Methods

### 5.3.1 Study sites

We examined variation in morphological characteristics (i.e. length, height and depth) of the major and minor claws of crabs on twelve sandy beaches with different levels of human disturbances on the South Carolina coast (Table 5.1). We conducted our field observations in July 2018. We chose study sites using the urbanization index (UI), modified from González et al. (2014), that was created based on observations and the quantitative evaluations during the summer of 2016 and 2017. During the summer of 2016 and 2017, we observed the beach cleaning technique, buildings, and other amenities next to each beach. We also counted the numbers of vehicles and beach visitors to obtain required data for creating the UI. Based on UI values, we grouped our study sites as pristine (P) sites, moderately impacted sites (MI), highly impacted sites by people (HI), and highly impacted sites by people and vehicles (HV). For a more detailed description of the study sites and the urbanization impacts on each, please see Gül & Griffen (in review(b)).

### 5.3.2. Claw morphology

To determine differences in claw size between male and female ghost crabs under various levels of human disturbance, we collected opportunistically-encountered individuals of a wide range of sizes around the strandline over approximately 1-km of beach during the night at each site. We measured the carapace width as well as the length, height, and depth of both the major (crusher) and minor (cutter) claws using a Vernier caliper ( $\pm 0.1$  mm) as shown in Fig. 5.1. We excluded individuals with injuries such as

missing limbs or injuries on the carapace. We used volumes of both claws (i.e. product of the claw dimensions, length X height X depth) as morphological claw characteristics of the crabs. We also used the variation in major claw height as a proxy for the claw closing force (Lee 1993, Yamada and Boulding 1998, Sneddon et al. 2000).

Because the claw dimensions are generally positively related to individual sizes in crustaceans, we controlled for crab size by regressing the log-transformed claw volumes and the major claw height against log-transformed carapace width of the crabs (Packard and Boardman 1999). We used the standardized residuals obtained from those regressions as response variables for all statistical analyses related to claw measurements. We used linear mixed effects models to examine whether claw volumes and the closing force varied between sexes, and sizes of the crabs and between levels of human disturbances. The residuals of the major and the minor claws volumes and the major claw height, after accounting for the crab size, were treated as the response variables in separate statistical analyses. For all statistical models, we used human disturbance level and sex and the size groups (i.e. juvenile vs adults) their interaction as categorical response variables. To control for spatial (latitudinal) differences associated with individual sites, we included the latitude of the study sites as a random factor in all mixed models. This was followed by a Tukey's HSD for multiple comparisons between sexes and disturbance levels ("lsmeans" package in R (Lenth & Hervé 2018)).

## **5.4 Results**

Relative volumes of the ghost crab major claws (the crusher claw) clearly varied between males and females based on the strength of human disturbance (Fig. 5.2).

Specifically, however the volume of the major claw was not different between male and female crabs at pristine sites, the male individuals had a larger crusher claw at moderately impacted sites and highly impacted sites by people and highly impacted sites by people and vehicles. Moreover, while the crusher volume of the males was similar across disturbance level, the variation in the volume of the crusher claw of the females varied with the strength of human disturbance (Table 5.2, Fig. 5.2).

The relative minor claw (the cutter claw) volume of the males was significantly lower than the minor claw volume of the females at pristine sites. At highly impacted sites by people, male individuals had larger residual minor claw volume. However, there was no difference in the minor claw volume between males and females at moderately impacted and highly impacted sites by people and vehicles (Table 5.2). Both male and female individuals had similar size of minor claws across disturbance levels (Table 5.2, Fig. 5.3).

The crusher claw closing strength of the males was higher compared to the claw closing strength of the females at pristine and at moderately impacted sites. However, there was no difference in the claw closing strength of the males and females at highly impacted sites (Table 5.2). Also, no difference was found in the claw closing strength of the crabs in the same sex related to the disturbance level (Table 5.2, Fig. 5.4).

## **5.5 Discussion**

We have shown that the relative length, height and depth of both the crusher and the cutter claws of *O. quadrata* change under various levels of human disturbance and that claw properties vary between males and females. We have further shown that the

height of the major claw, a proxy for the closing strength of the claws in crabs (Lee 1993, Yamada & Boulding 1998, Sneddon et al. 2000), was lowest in females at moderately impacted sites and in males at sites that were highly disturbed by people and vehicles. Since claw size and claw closing strength can directly influence sexual selection (Shine 1989, Claverie & Smith 2010, Cothran & Jeyasingh 2010), foraging efficiency (Yamada & Boulding 1998, Edgel & Rochette 2009, Jaroensutasinee & Jaroensutasinee 2015), success in agonistic behaviors (Sneddon et al. 2000, Shimoda et al. 2000, Cannizzo et al. 2019, reviewed in Rico-Guevera & Hurme 2019), and mating success (Serrano-Meneses et al. 2007), changes in the claw morphology have the potential to influence the ecology and the physiology of *O. quadrata*. The results of this research therefore have important implications for ghost crab populations on sandy shores and for our understanding of the broad potential influences of human disturbances on the ecological and physiological changes of organisms.

The changes in the claw characteristics as a response to the levels of the human disturbance reported here may reflect variation in the strength of the agonistic behaviors under human influence. Agonistic behaviors are widespread in crustaceans (Dalosto et al. 2013, Ayres-Peres et al. 2015), which commonly use claws as an important weapon for winning these encounters (Anderson 1994, Rico-Guevera & Hurme, 2019), and ghost crabs are no exception (Lucrezi & Schlacher 2014). The strength of agonistic behaviors is directly related to density in crustaceans, and individuals living at higher population densities often become better competitors, likely due to selection for stronger claws used in agonistic encounters (Calsbeek 2009). Size of the crusher claws varied between males and females across levels of human disturbance, suggesting that there may be a stronger

selection for female crabs on sandy shores in terms of claw characteristics. Specifically, claw characteristics, relative to their body size (e.g. carapace width), of both male and female crabs decline proportionately with increasing strength of human disturbance. As a bioindicator of human disturbance on the sandy shores, population abundance and the individual sizes of Atlantic ghost crabs decline dramatically under the influence of human disturbance (Wolcott & Wolcott 1984, Neves & Bemvenuti 2006, Hobbs et al. 2008, Gül & Griffen 2018a, 2018b, Suici et al. 2018). Thus, there are potentially weaker agonistic behaviors at sites under strong human influences. Moreover, the lowest claw closing strength (i.e., claw height) was found in crabs living at the sites in which the human disturbance is strongest (e.g. at highly impacted sites by people and vehicles). Previous work has shown that the ghost crab density was lowest in those sites (Gül & Griffen 2018a, 2018b).

Results related to the relative size of both claws here may also be a result of variation in the available energy for crabs living in sites with various levels of human disturbance. Specifically, both male and female crabs have much smaller crusher and cutter claws at beaches under human influence compared to their conspecifics at pristine sites. Besides the decrease in the ghost crab populations, human disturbance dramatically influences the main prey items of ghost crabs such as bean clams, mole crabs and sandy beach coleoptera (González et al. 2014, Cardoso et al. 2016, Frota et al. 2019, Laitano et al. 2019). Diet type and overall resource availability can influence the claw morphology and size in other species (Shine 1989, Smith & Palmer 1994, Cothran & Jeyasingh 2010). If this is true for ghost crab populations, it is logical to conclude that reductions in preferred food lead to smaller claw sizes in ghost crabs at disturbed sites. However, this

explanation raises the question of why limited food would not influence male crabs in the same way that it does the females? Hartnoll (1974) reported that male crabs grow their claws faster compared to the females after reaching maturity. Similar results have been reported for ghost crabs. Haley (1969) showed that claws of male ghost crabs grow faster compared to those of females at all size classes. This differential growth may result from sexual selection (Shenk & Wainwright 2001). Therefore, it can be hypothesized that male ghost crabs grow their claws faster even under human disturbance to attract females, as fiddler crabs do (i.e. fiddler crabs and ghost crabs are the members of the same family, Ocypodidae) (McLain & Pratt 2007, Reaney 2009), or for use in mate guarding. Overall, the results here show that human disturbance influences the claw size of both male and female crabs; however, the impact is more drastic for females.

Finally, we only examined variation in claw size and did not directly measure claw closing strength. However, previous studies have shown that there is a close relationship between claw size (e.g. claw height) and the closing strength across species (Lee 1993, Yamada & Boulding 1998, Sneddon et al. 2000), as well as between claw size and resource availability and foraging efficiency (Smith & Palmer 1994, Yamada & Boulding 1998, Buck et al. 2003, Edgell & Rochette 2009, Cothran & Jeyasingh 2010). Ghost crabs mostly feed on beach clams and mole crabs on the Southern coast (Wolcott 1978), and these organisms are easy to open and/or to crush for a crab with even a weak claw, suggesting that the dietary implications of reduced claw size in this system may be moderate. Further research is required to understand whether the lower claw height and closing strength influence the diet of ghost crabs.



The direct link between the level of human disturbance and claw size of ghost crabs suggests that human disturbances may have a negative influence on these natural populations. As is mentioned above, ghost crabs, as a common bioindicator species of human influences, show smaller individual sizes and lower abundances at the sites with human disturbance (Wolcott & Wolcott 1984, Neves & Bemvenuti 2006, Hobbs et al. 2008, Schlacher et al. 2016, Gül & Griffen 2018a, 2018b, Suicu et al. 2018) similar to the other bioindicator species in various ecosystems (Carignan & Villard 2002, Spellerberg 2005 Heink & Kowarik 2010, Siddig et al. 2016). However, the mechanistic reason for these responses of bioindicators remain unclear. The smaller claw size of males and females (independent of body size) under human disturbance compared to claw sizes of crabs at pristine sites may partially reflect the mechanistic driver of reduced overall body size of this species due to human disturbance. If the available energy sources are limited under human influence as hypothesized above, then species may not obtain enough energy at the most disturbed sites. In that case, both males and females may exhibit a trade-off between their growth and their reproductive activity. Females may spend more of the energy that they gain for egg production instead of claw growth, while males may spend more energy for increasing their chance of gaining access to a female by growing their claws instead of their body. Further studies are required to test this idea by investigating the differences in daily activities (e.g. burrow size and behavior) between males and females, as well as differences in the energy requirement for reproduction and for claw growth.

In conclusion, human disturbance plays a key role in determining the size of the major and minor claws of a bioindicator species, *O. quadrata*. One plausible reason for

this is a shortage in energy sources, which would simultaneously explain the reported decrease in overall body size on beaches influenced by human disturbance. However, the potential influences of the smaller claw size on the foraging efficiency and success in mating and in agonistic encounters remain to be tested. Understanding the possible influences of such morphological variations on the ecology and the physiology of individuals will further our understanding of population level responses of this bioindicator species to various levels of human disturbance.

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## 5.7 Tables

Table 5.1. Urbanization indicators from absent (0) to extremely high (5) and urbanization index (UI) values of the study sites. The sites were aligned from north to south (A=proximity to urban centers, B = buildings on the sand and dunes, C= beach cleaning, D= number of vehicles on the sand, E= frequency of visitors, F= infrastructures).

Site	Impact	Latitude Longitude	A	B	C	D	E	F	UI
Waties Island 1	P	33°50'52" N 78°33'48" W	0	0	0	0	1	0	0.03
Waties Island 2	P	33°50'45" N 78°34'33" W	0	0	0	0	1	0	0.03
N. Myrtle Beach	HV	33°48'50" N 78°40'49" W	5	4	5	4	5	5	0.93
Myrtle Beach	HV	33°40'01" N 78°54'21" W	5	4	5	5	5	5	0.96
Garden City Beach	HV	33°34'13" N 79°00'11" W	5	5	5	3	5	4	0.9
Pawley's Island	MI	33°25'49" N 79°07'12" W	3	4	0	0	2	2	0.36
Debidue Island	P	33°21'15" N 79°09'08" W	1	0	0	0	1	0	0.06
Isle of Palm 2	HI	32°47'49" N 79°45'09" W	5	4	3	2	4	3	0.7
Isle of Palm 1	MI	32°46'29" N 79°48'28" W	3	3	0	0	2	1	0.3
Sullivan's Island 1	HI	32°45'43" N 79°49'32" W	4	3	3	3	4	3	0.66
Sullivan's Island 2	HI	32°45'23" N 79°50'24" W	4	3	3	3	4	3	0.66
Folly Beach	MI	32°39'33" N 79°55'36" W	4	4	1	0	3	3	0.5

Table 5.2. Summary of linear mixed effects model (LMER) showing the residual claw volumes and closing strength of ghost crabs after accounting for crab size under various levels of human disturbance compared to the pristine sites (A = moderately impacted sites, B = highly impacted sites by people, C = highly impacted sites by people and vehicles, D = sex). Latitudes of the study sites were included to model as random effect.

<b>Fixed factor</b>	<b>S.E.</b>	<b><i>t</i>-value</b>	<b><i>p</i>-value</b>
<b>Major claw volume</b>			
<i>A (moderate impact)</i>	0.061	-3.848	0.007
<i>B (high impact by people)</i>	0.071	-1.904	0.113
<i>C (high impact by people and vehicles)</i>	0.06	-3.22	0.019
<i>D (sex)</i>	0.016	0.322	0.747
<i>A X D</i>	0.029	8.303	<0.001
<i>B X D</i>	0.038	2.67	0.007
<i>C X D</i>	0.038	3.766	0.0001
<b>Minor claw volume</b>			
<i>A</i>	0.06	-3.836	0.007
<i>B</i>	0.08	-3.091	0.017
<i>C</i>	0.089	-3.523	0.009
<i>D</i>	0.021	-3.679	0.0002
<i>A X D</i>	0.038	7.173	<0.001
<i>B X D</i>	0.049	5.032	<0.001
<i>C X D</i>	0.049	1.501	<0.001
<b>Claw closing force</b>			
<i>A</i>	0.024	-3.233	0.015
<i>B</i>	0.027	-1.467	0.183
<i>C</i>	0.025	-2.256	0.056
<i>D</i>	0.007	8.542	<0.001
<i>A X D</i>	0.012	4.388	<0.001
<i>B X D</i>	0.016	-1.829	0.068
<i>C X D</i>	0.016	-3.085	0.0022

## 5.8 Figures

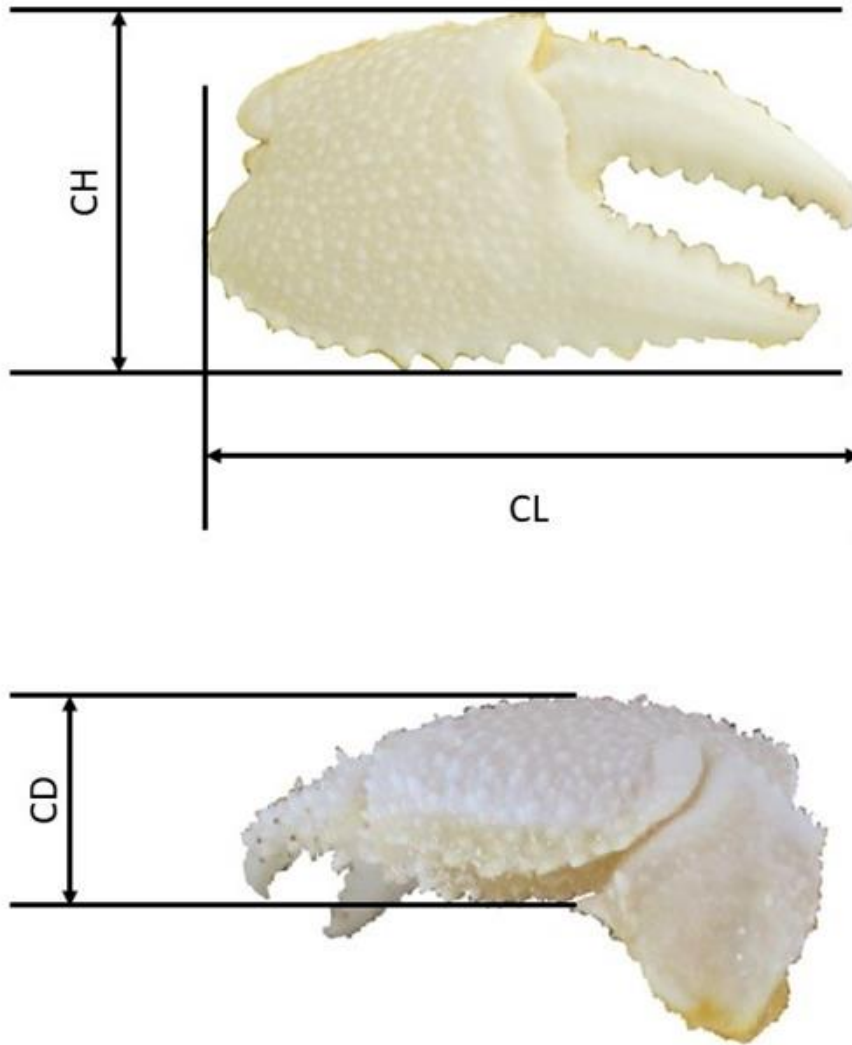


Figure 5.1. Claw dimensions measured for ghost crabs, *O. quadrata* (CL = claw length, CH = claw height, CD = claw depth).

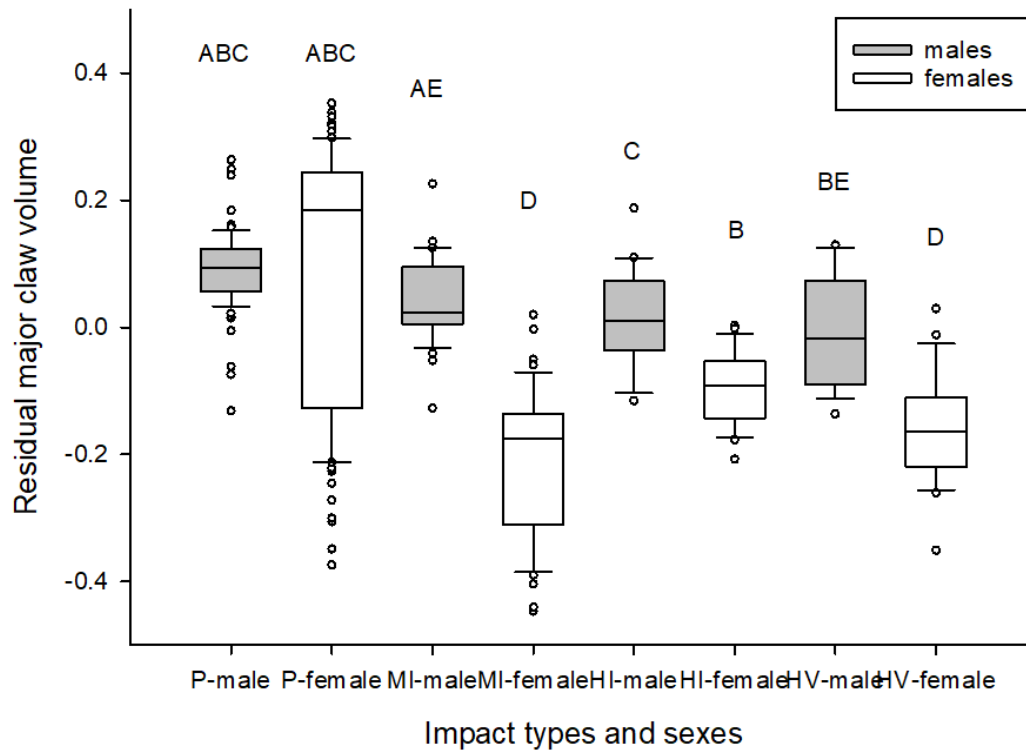


Figure 5.2. Variation in residual major claw volume for *O. quadrata* among various levels of human disturbance on South Carolina beaches. Capital letters within graph represent significant differences based on Tukey's HSD. Horizontal lines indicate the median value at each disturbance level, boxes encompass the upper and lower quartiles of the data, whiskers encompass 95% of the data, and circles are outliers that fall outside of this range.

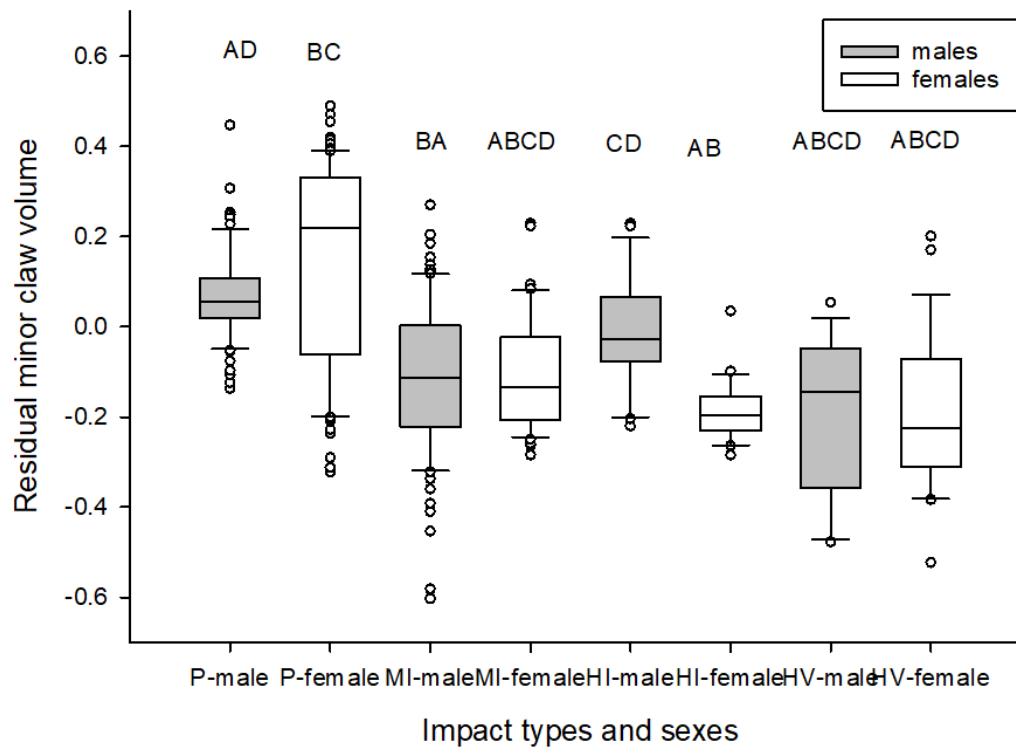


Figure 5.3. Variation in residual minor claw volume for *O. quadrata* among various levels of human disturbance on South Carolina beaches. Capital letters within graph represent significant differences based on Tukey's HSD. Symbols and letters are as described in the legend for Fig 2.

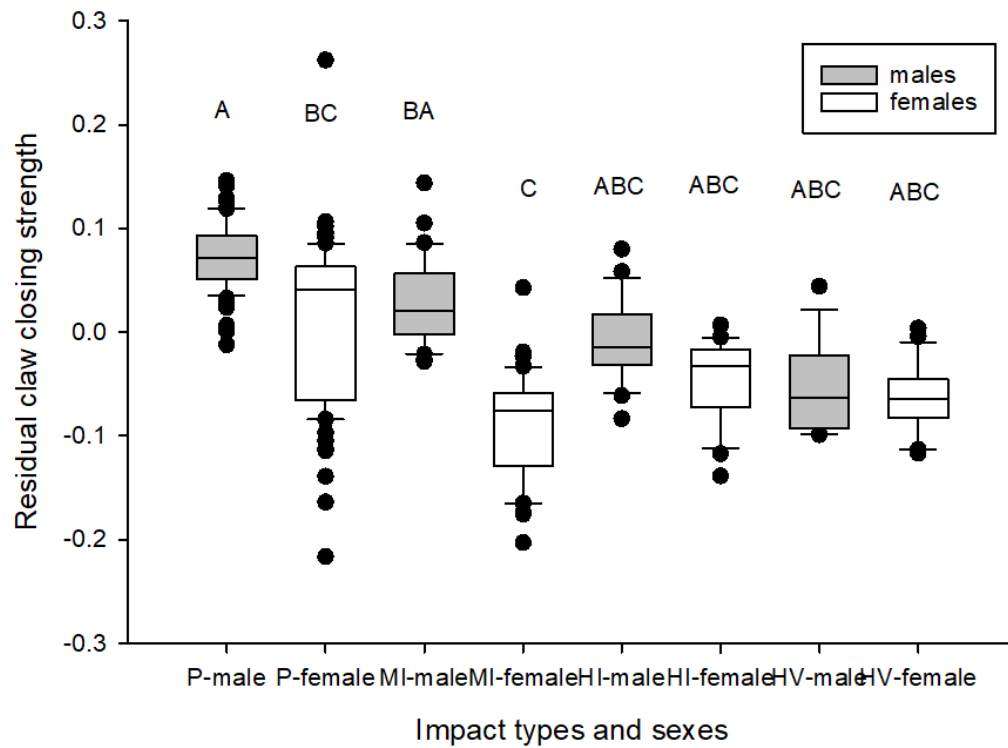


Figure 5.4. Variation in residual claw closing force for *O. quadrata* among various levels of human disturbance on South Carolina beaches. Capital letters within graph represent significant differences based on Tukey's HSD. Symbols and letters are as described in the legend for Fig 2.



## 5.9. Literature Cited

- Andersson, M. (1994). *Sexual Selection*. Princeton, New Jersey. Princeton University Press
- Ayres-Peres, L., P. B. Araujo, C.G. Jara, A.V. Palaoro & Santos, S. (2015). How variable is agonistic behavior among crab species? A case study on freshwater anomurans (Crustacea: Decapoda: Aeglidae). *Journal of Zoology*, 297(2):115-122.  
<https://doi.org/10.1111/jzo.12262>
- Barbier, E.B., S.D. Hacker, C. Kennedy, E.W. Koch, E.C. Siter & Silliman, B.R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81(2):169–193. <https://doi.org/10.1890/10-1510.1>
- Buck, T.L., G.A. Breed, S.C. Pennings, M.E. Chase, M. Zimmer & Carefoot, T.H. (2003). Diet choice in an omnivorous salt-marsh crab: different food types, body size, and habitat complexity. *Journal of Experimental Marine Biology and Ecology*, 292:103-116.
- Calsbeek, R. (2009). Experimental evidence that competition and habitat use shape the individual fitness surface. *Journal of Evolutionary Biology*, 22:97–108.  
[doi:10.1111/j.1420-9101.2008.01625.x](https://doi.org/10.1111/j.1420-9101.2008.01625.x)
- Cannizzo, Z.J., S.K. Nix, I.C. Whaling & Griffen, B.D. (2019). Individual morphology and habitat structure alter social interactions in a range-shifting species. *Diversity*, 11:6.  
[doi:10.3390/d11010006](https://doi.org/10.3390/d11010006)

Cardoso, R.S., C.A.M. Barboza, V.B. Skinner & Cabrini, T.M.B. (2016). Crustaceans as ecological indicators of metropolitan sandy beach health. *Ecological Indicators*, 62:154-162. <https://doi.org/10.1016/j.ecolind.2015.11.039>

Carignan, V. & Villard, M.A. (2002). Selecting indicator species to monitor ecological integrity: a review. *Environmental Monitoring and Assessment*, 78(1):45-61.  
PMID:12197640

Claverie, T. & Smith, I.P. (2010). Allometry and sexual dimorphism in the chela shape in the squat lobster *Munida rugosa*. *Aquatic Biology*, 8:179-187. doi: 10.3354/ab00233

Clutton-Brock, T. (2017). Reproductive competition and sexual selection. *Philosophical Transactions of the Royal Society B*, 372:20160310.  
<http://dx.doi.org/10.1098/rstb.2016.0310>

Cortes, R.M.V., S.J. Hughes, V.R. Pereira & Varandas, S.D.P. (2013). Tools for bioindicator assessment in rivers: the importance of spatial scale, land use patterns and biotic integration. *Ecological Indicators* 34:460-477.  
<https://doi.org/10.1016/j.ecolind.2013.06.004>

Cothran, R.D. & Jeyasingh, P.D. (2010). Condition dependence of a sexually selected trait in crustacean species complex: importance of the ecological context. *Evolution*, 64(9):2535-25346. doi:10.1111/j.1558-5646.2010.00998.x

Dalosto, M.M., A.V. Palaoro, J.R. Costa & Santos, S. (2013). Aggressiveness and life underground: the case of burrowing crayfish. *Behaviour*, 150:3-22.  
DOI:10.1163/1568539X-00003034

- Davenport, J. & Davenport, J.L. (2006). The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine, Coastal and Shelf Science*, 67(1-2):280-292
- Defeo, O., A. McLachlan, D.S. Schoeman, T.A. Schlacher, J. Dugan, A. Jones, M. Lastra & Scapini, F. (2009). Threats to sandy beach ecosystems: A review. *Estuarine, Coastal and Shelf Science*, 81(1):1- 12. <https://doi.org/10.1016/j.ecss.2008.09.022>
- Edgell, T.C. & Rochette, R. (2009). Prey-induced changes to a predator's behaviour and morphology: Implications for shell–claw covariance in the northwest Atlantic. *Journal of Experimental Marine Biology and Ecology*, 382:1-7. doi:10.1016/j.jembe.2009.10.004
- Fisher, J. & Tevesz, M. (1979). Within-habitat spatial patterns of *Ocypode qudrata* (Fabricius) (Decapoda Brachyura). *Crustaceana*, 5:31–36
- Frota, G.P., T.M.B. Cabrini & Cardoso, R.S. (2019). Fluctuating asymmetry of two crustacean species on fourteen sandy beaches of Rio de Janeiro State. *Estuarine, Coastal and Shelf Science*, 223:138-146. <https://doi.org/10.1016/j.ecss.2019.03.013>
- Giri, F. & Loy, A. (2008). Size and shape variation of two freshwater crabs in Argentinean Patagonia: the influence of sexual dimorphism, habitat, and species interactions. *Journal of Crustacean Biology*, 28(1):37-45
- González, S.A., K. Yáñez-Navea & Muñoz, M. (2014). Effect of coastal urbanization on sandy beach coleoptera *Phaleria maculate* (Kulzer, 1959) in northern Chile. *Marine Pollution Bulletin*, 83(1):265–274. <https://doi.org/10.1016/j.marpolbul.2014.03.042>

Gül, M.R. & Griffen, B.D. (2018a). A reliable bioindicator of anthropogenic impact on the coast of South Carolina. *Southeastern Naturalist*, 17(2):357–364.

<https://doi.org/10.1656/058.017.0217>

Gül, M.R. & Griffen, B.D. (2018b). Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores. *PLoS ONE*, 13(12):e0209977.

<https://doi.org/10.1371/journal.pone.0209977>

Gül, M.R. & Griffen, B.D. (2019). Combined impacts of natural and anthropogenic disturbances on a bioindicator species. *Journal of Experimental Marine Biology and Ecology*, 519: 151185. <https://doi.org/10.1016/j.jembe.2019.151185>

Gül, M.R. & Griffen, B.D. (in review (a)). Human impacts change diet, energy storage, and reproduction in bioindicator species. *Journal of Animal Ecology*

Gül, M.R. & Griffen, B.D. (in review (b)). Burrowing behavior and burrowing energetics of a bioindicator under human disturbance. *Ecology and Evolution*.

Haley, S.R. (1969). Relative growth and sexual maturity of the Texas ghost crab, *Ocypode quadrata* (Fabr.). (*Brachyura*, *Ocypodidae*). *Crustaceana*, 17:285–297

Halpern, B.S., S. Walbridge, K.A. Selkoe, C.V. Kappel, F. Micheli, C. D’Agros, J. Bruno, K.S. Casey, C. Ebert, H.E. Fox, R. Fujita, D. Heinemann, L.S. Lenihan, E.M.P. Madin, M.T. Perry, E.R. Selig, M. Spalding, R. Steneck & Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, 319:948–952.  
<https://doi.org/10.1126/science.1149345>

- Hartnoll, R.G. (1974). Variation in growth pattern between some secondary sexual characters in crabs (Decapoda Brachyura). *Crustaceana*, 27:131–136
- Heink, U. & Kowarik, I. (2010). What are indicators? On the definition of indicators in ecology and environmental planning. *Ecological Indicators*, 10(3):584–593.  
doi:10.1016/j.ecolind.2009.09.009
- Hernández, P. & João, M.C.A. (2018). Social structure, sexual dimorphism and relative growth in the ghost shrimp *Callichirus seilacheri* (Bott, 1955) (Decapoda, Axiidea, Callianassidae) from the tropical eastern Pacific. *Marine Biology Research*, 14(8):856-867. <https://doi.org/10.1080/17451000.2018.1529415>
- Hobbs, C.H., C.B. Landry & Perry, J.E. (2008). Assessing anthropogenic and natural impacts on ghost crabs (*Ocypode quadrata*) at Cape Hatteras National Seashore, North Carolina. *Journal of Coastal Research*, 24(6):1450–1458. <https://doi.org/10.2112/07-0856.1>
- Jaroensutasinee, F.W.T.M. & Jaroensutasinee, K. (2015). Effects of sexual dimorphism and body size on feeding behaviour of the fiddler crab, *Uca bengali* Crane, 1975. *Crustaceana*, 88(2):231-242. DOI 10.1163/15685403-00003405.
- Laitano, M.V., N.M. Chiaradia & Nuñez, J.D. (2019). Clam population dynamics as an indicator of beach urbanization impacts. *Ecological Indicators*, 101:926-932.  
<https://doi.org/10.1016/j.ecolind.2019.02.006>
- Lee, S.Y. (1993). Chela height is an acceptable indicator of chela strength in *Carcinus maenas* (Linnaeus, 1758) (Decapoda, Brachyura). *Crustaceana*, 65:115-116

Length, R.V. & Hervé, M. (2018). Package "lsmeans" <https://cran.r-project.org/web/packages/lsmeans/lsmeans.pdf>

Lucrezi, S. & Schlacher, T.A. (2014). The ecology o ghost crabs-a review. *Oceanography and Marine Biology- Annual Review*, 52:201-256

Maklakov, A.A., T. Bilde & Lubin, Y. (2004). Sexual selection for increased male body size and protandry in a spider. *Animal Behaviour*, 68(5):1041-1048.  
<https://doi.org/10.1016/j.anbehav.2004.02.010>

McLain, D.K. & Pratt, A.E. (2007). Approach of females to magnified reflections indicates that claw size of waving fiddler crabs correlates with signaling effectiveness. *Journal of Experimental Marine Biology and Ecology*, 343(2):227-238.  
<https://doi.org/10.1016/j.jembe.2006.12.011>

Milne, L.J. & Milne, M.J. (1941). Notes on the behavior of the ghost crab. *American Naturalist*, 80:362–380.

Neff, B.D. & Svensson, E.I. (2013). Polyandry and alternative mating tactics. *Philosophical Transactions of the Royal Society B*, 368:20120045.  
<http://dx.doi.org/10.1098/rstb.2012.0045>

Neves, F.M. & Bemvenuti, C.E. (2006). The ghost crab *Ocypode quadrata* (Fabricius, 1787) as a potential indicator of anthropogenic impact along the Rio Grande do Sul coast, Brazil. *Ecological Indicators*, 133(4): 431–435.  
<https://doi.org/10.1016/j.biocon.2006.04.041>

- Nolen, Z.J., P.E. Allen & Miller, C.W. (2017). Seasonal resource value and male size influence male aggressive interactions in the leaf footed cactus bug, *Narnia femorata*. *Behavioural Processes*, 138:1–6.
- Packard, G.C. & Boardman, T.J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comparative Biochemistry and Physiology B*, 122:37-44.
- Pombo, M. & Turra, A. (2013). Issues to be considered in counting burrows as a measure of Atlantic ghost crab populations, an important bioindicator of sandy beaches. *PLoS ONE*, 8(12):e83792. <https://doi.org/10.1371/journal.pone.0083792>
- Reaney, L.T. (2009). Female preference for male phenotypic traits in a fiddler crab: do females use absolute or comparative evaluation? *Animal Behaviour*, 77:139–143. <https://doi.org/10.1016/j.anbehav.2008.09.019>
- Rico-Guevara, A. & Hurme, K.J. (2019). Intrsexually selected weapons. *Biological Reviews*, 94:60-101. doi: 10.1111/brv.12436
- Serrano- Meneses, M.A., A. Córdoba-Aguilar, V. Méndez, J. Layen & Székely, T. (2007). Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. *Animal Behaviour*, 73:987-997. doi:10.1016/j.anbehav.2006.08.012
- Schenk, S.C. & Wainwright, P.C. (2001). Dimorphism and the functional basis of claw strength in six brachyuran crabs. *Journal of Zoology*, 255:105-119.

- Schlacher, T.A., S. Lucrezi, R.M. Connolly, C.H. Peterson, B.L. Gilby, B. Maslo, A.D. Olds, S.J. Walker, J.X. Leon, C.M. Huijbers, M.A. Weston, A. Turra, G.A. Hyndes, R.A. Holt & Schoeman, D.S. (2016). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal and Shelf Science*, 169:56-73. <https://doi.org/10.1016/j.ecss.2015.11.025>
- Schlacher, T.A., L.M.C. Thompson & Price, S. (2007). Vehicles versus conservation of invertebrates on sandy beaches: quantifying direct mortalities inflicted by off-road vehicles (ORVs) on ghost crabs. *Marine Ecology*, 28:354–367
- Shine, R. (1989). Ecological causes for the evolution of sexual size dimorphism: a review of the evidence. *The Quarterly Review of Biology*, 64:419-461
- Siddig, A.A.H., A.M. Ellison, A. Ochs, C. Villar-Leeman & Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in ecological indicators. *Ecological Indicators*, 60:223-230. doi:10.1016/j.ecolind.2015.06.036
- Smith, L.D. & Palmer, A.R. (1994). Effects of manipulated diet on size and performance of Brachyuran crab claws. *Science*, 264:710-712.
- Sneddon, L.U., F.A. Huntingford, A.C. Taylor & Orr, J.F. (2000). Weapon strength and competitive success in the fights of shore crabs (*Carcinus maenas*). *Journal of Zoology*, 250:397- 403
- Spellerberg, I. (2005). *Monitoring ecological change* (2nd ed.). Cambridge, UK: Cambridge University Press



- Stelling-Wood, T.P., G.F. Clark, & Poore, A.G.B. (2016). Responses of ghost crabs to habitat modification of urban sandy beaches. *Marine Environmental Research*, 116:32–40. <https://doi.org/10.1016/j.marenvres.2016.02.009>
- Suciu, M.C., D.C. Tavares & Zalmon, I.R. (2018). Comparative evaluation of crustaceans as bioindicators of human impact on Brazilian sandy beaches. *Journal of Crustacean Biology*, ruy027. <https://doi.org/10.1093/jcbiol/ruy027>
- Vitousek, P.M., H.A. Mooney, L. Lubchenco & Melillo, J.M. (1997). Human domination of Earth's ecosystems. *Science* 277:494–499. <https://doi.org/10.1126/science.277.5325.494>
- Wolcott, T.G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fab.) on an ocean beach: scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31:67–82.
- Wolcott, T.G. & Wolcott, D.L. (1984). Impact of off-road vehicles on macroinvertebrates of a mid- atlantic beach. *Biological Conservation*, 29(3):217-240
- Yamada, S.B. & Boulding, E.G. (1998). Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. *Journal of Experimental Marine Biology and Ecology*, 220:191-211

CHAPTER 6

BURROWING BEHAVIOR AND BURROWING ENERGETICS OF A  
BIOINDICATOR UNDER HUMAN DISTURBANCE<sup>1</sup>

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<sup>1</sup>Gül MR. & Griffen BD. Burrowing behavior and burrowing energetics of a bioindicator under human. Submitted to *Ecology and Evolution*.

## 6.1 Abstract

Bioindicator species are extensively used for rapid assessment of ecological changes. Their use commonly focuses on changes in population abundance and individual sizes in response to environmental change. These numerical and demographic shifts likely have behavioral and physiological mechanistic drivers that, if understood, could provide additional insights into the use of these species as bioindicators of habitat health. The Atlantic ghost crab, *Ocypode quadrata*, is a global bio-indicator species of human disturbance on sandy shores. Individual size and population abundance of *O. quadrata* decline dramatically at sites with human disturbance, and the cause of this phenomenon remains unclear. Here, we test the hypothesis that individual and population-level changes at disturbed sites reflect changes in burrowing behavior and energetics. Specifically, we examine whether or not the burrowing behavior (e.g. burrow fidelity and longevity) of *O. quadrata* changes because of human disturbance. We also examine energy required for burrowing by *O. quadrata* across different levels of human disturbance. We show that *O. quadrata* has the highest burrow fidelity and longevity at sites with low level of human impact, and weakest burrow fidelity and longevity at pristine sites. *O. quadrata* reduce the burrowing energy allocation by manipulating the burrow dimension and increasing the burrow longevity even under low levels of human disturbance. Overall, this study shows that human disturbances not only change the behavior of organisms, but also shift energetic balance. Our results support the use of a bioenergetic approach to better understand how human disturbances influence natural populations, and the specific use of this approach with this bioindicator species.

## 6.2 Introduction

Bioindicator species are widely used to identify and measure human-related ecological changes (McGeoch 1998, Carignan & Villard 2002, Spellerberg 2005, Siddig et al. 2016) in many different ecosystems from forests (Rainio & Niemelä 2003, Pearce & Venier 2006, Maleque et al. 2009) to coral reefs (Erdmann & Caldwell 1997, Hallock et al. 2003). The main reason for using bioindicators is the reduced cost, time, and effort compared to examining all biota in a disturbed region (Carignan & Villard 2002, Spellerberg 2005, Cortes et al. 2013). Thus, the species that is selected as an indicator should represent the ecological changes in a given area (Carignan & Villard 2002, Siddig et al. 2016), which is particularly important in coastal regions due to the difficulty of assessing the impacts of human disturbances in marine environments (Vitousek et al. 1997) because of their highly dynamic nature (Carr et al. 2003).

Bioindicator species usually signal changes in a given ecosystem via changes in their presence/absence, abundance, or age/size structure (Carignan & Villard 2002, Spellerberg 2005, Heink et al. 2016). However, changes in behavior may also be used as a bioindicator, as species may change their behavior and daily activities under altered conditions due to direct and indirect human disturbances (Sih et al. 2010, Sih 2013, Wong & Candolin 2015, Fontúrbel et al. 2015, Costa et al. 2018). For example, population abundance of some crab species are commonly used as bioindicators of various human disturbances such as urbanization, mining, and contamination (Cannicci et al. 2009, Wildsmith et al. 2009, Jonah et al. 2015, Schlacher et al. 2016a), and some of these species additionally alter burrowing behaviors in disturbed sites (Weis & Perlmutter 1987, Culbertson et al. 2007, Bartolini et al. 2009, Gül & Griffen 2018a). Species may

also alter their feeding habits (Jokimäki et al. 2016, Griffiths et al. 2017) and trophic interactions (Gray et al. 2007, Costa et al. 2017) in the areas with human disturbance, and thus these changes in behaviors and daily activities can result in changes to the energy balance and physiological state (Spellerberg 2005, Chandurvelan et al. 2015). Therefore, despite the fact that studies on the energetic results of human disturbance in bioindicator species are limited to a few examples (Toro et al. 2011), there are several examples to show that alterations in the physiological/energetic conditions of non-bioindicator organisms are due to direct (Williams et al. 2006, Symons et al. 2014, Villegas-Amtmann et al. 2015) and indirect human impacts (Thomas et al. 2016, Griffen 2018, Leo et al. 2018).

Burrowing behavior is a common phenomenon in organisms (Reichman & Smith 1990, Nomura et al. 2009, Lucrezi & Schlacher 2014). Despite its energetically expensive nature (Hunter & Elder 1989), burrowing behavior provides some advantages, including protection from predators and cannibals, from direct human disturbance, and from harsh environmental conditions (Soriguer & Amat 1980, Christoffers 1986, Friend, 1993; Lucrezi & Schlacher 2014, Gül & Griffen 2018a). Burrowing species commonly use olfactory and visual cues to return to their burrows (Hughes 1966, Bonadonna et al. 2001, Ribeiro et al. 2006, Lucrezi & Schlacher 2014) and as a result, show high fidelity to individual burrows and sites.

Intensity of human disturbance on sandy beaches generally increases with human population size (Davenport & Davenport 2006, Halpern et al. 2008, Defeo et al. 2009). The ecological impacts of this disturbance are commonly assessed using the population abundance and size structures of common macroinvertebrates such as clams (Defeo & de

Alava 1995, Schlacher et al. 2008, Sheppard et al. 2009), mole crabs and sand hoppers (Cardoso et al. 2016), beetles (González et al. 2014), and ghost crabs (Steiner & Leatherman 1981, Wolcott & Wolcott 1984, Barros 2001, Neves & Bemvenuti 2006, Hobbs et al. 2008; Lucrezi et al. 2009, Aheto et al. 2011, Schlacher et al. 2016a, Gül & Griffen 2018a, 2018b).

The most widespread responses of ghost crabs to human disturbances on sandy shores are decreases in population abundance and individual body sizes (Schlacher et al. 2016a and citations therein, Gül & Griffen 2018a, 2018b). These changes are often measured using burrow counts, a non-destructive and efficient technique for assessing both the abundance and size distribution of ghost crabs (Schlacher et al. 2016a). Various mechanisms have been hypothesized for the reduced density and size of ghost crabs with human disturbance, including higher mortality rate for individuals with shallow burrows (Schlacher et al. 2007), direct crushing by vehicles (Wolcott & Wolcott 1984), lower organic material availability (Stelling-Wood et al. 2016), and stress due to direct handling by people (Gül and Griffen 2018a). However, the precise mechanistic reason for observed demographic changes remains unclear. Besides these demographic changes, ghost crabs also alter their burrow architecture under human influence (Lucrezi & Schlacher 2010, Schlacher & Lucrezi 2010, Gül and Griffen 2018a), implying that there may be an energetic component to the response of ghost crabs to disturbance. Here we investigate whether Atlantic ghost crabs, *Ocypode quadrata*, show any variation in the burrowing behavior (fidelity and longevity) and energy demand for burrowing activities between sites with different levels of human disturbance. We predicted that *O. quadrata* will be forced to burrow more frequently on disturbed sites because their burrows would

be destroyed by human activities more often compared to pristine sites. We further predicted that burrowing would represent an energetically expensive behavior and that this energetic cost should be influenced by changes in the frequency of burrows and the size of burrows across beaches with different levels of human disturbance.

## **6.3 Materials and Methods**

### *6.3.1 Study system*

We examined the burrowing behavior of the Atlantic ghost crab, *O. quadrata*, on twenty South Carolina sandy beaches with different levels of human disturbances between 15<sup>th</sup> May and 1<sup>st</sup> November 2017 (Table 6.1). We classified and grouped our study sites by using the urbanization index (UI) that was modified from González et al. (2014). Specifically, we used the following 6 variables based on observations and counts during the summer of 2016 and 2017 to estimate urbanization levels: (1) proximity to urban centers, (2) building on the sand and dunes, (3) beach cleaning, (4) number of vehicles on the sand, (5) visitor frequency, and (6) infrastructure such as parking lots, restrooms, and other amenities. Variables 3, 4, and 5 were obtained by direct counts during our study. Since beach cleaning is performed during the nights, we observed whether the beach was mechanically cleaned and counted the number of vehicles on the beach during the night. For frequency of visitors, we counted all visitors for two hours in the morning between 09:00 and 11:00 as a proxy. On the other hand, levels 1, 2, and 6 were acquired by direct observations. We scored each level from “0” to “5” based on the level of estimated variables (Table B.1, Supporting information Appendix 1). We then summed these scores across the six variables described above and divided by 30,

providing a UI score that ranged from 0 to 1. Finally, we grouped our study sites by using UI scores as pristine (P: from 0 to 0.25), moderately impacted by only visitors (MI: from 0.26 to 0.50), highly impacted by only visitors (HI: from 0.51 to 0.75), and highly impacted by human visitors and vehicles (HV: from 0.76 to 1).

### *6.3.2 Impact of marking procedure*

We used a mark-recapture study to examine burrow fidelity (described below). Prior to this, we used a preliminary study to determine whether our mark and recapture technique would likely alter burrowing behavior (e.g. burrow longevity) of ghost crabs. This preliminary experiment did not determine whether our procedure influenced the burrow fidelity of crabs. We therefore make the assumption that any impacts of our procedures on burrow fidelity did not differ across levels of human impact. We selected one site for each human disturbance level. At each site, we selected newly-created burrows in the upper zone (i.e. on the seaward side of the dune vegetation) and designated them as control or treatment burrows. Burrows were identified as “newly-created” by initially marking existing burrows and then selecting new burrows for the study as they were created by crabs. We examined a total of 84 burrows (i.e. 42 control, 42 treatment) across the four sites. Each burrow was marked using a marking flag. We were forced to bury our marking flags in the sand at about a 20-cm depth in the sites with human presence because beachgoers removed unburied flags. For the control burrows, we used a set of burrows that were newly-created over a three-day period and examined these until they collapsed to determine the burrowing behavior and to measure burrow longevity. For the treatment burrows, we set up simple traps (buckets buried flush with the sand and with a piece of rotting fish meat to lure crabs into the buckets)



before sunset to capture the crabs that newly created their burrows. We also surrounded burrows and traps together using plastic mesh to force the crabs into the traps. We were careful not to destroy the sand mounds and not to remove any material (e.g. small stones) from around the burrows, as individuals use sand mounds (Hughes 1966, Lucrezi & Schlacher 2014) and marine debris (Costa et al. 2018) as visual cues to recognize their own burrow. All captured crabs in the buckets were marked on the ventral side using nail polish, which is visible for up to 2 months in this species (Christoffers 1986). After we completed our marking procedure, we removed the traps and mesh around the burrows. We applied the same marking procedure for all newly-created burrows and crabs for the next three days. To ensure that an individual still owned the same burrow, we applied the same trapping technique every three days until the burrow collapsed. We used separate GLMs with a Poisson distribution for each site to understand whether the number of days during which the burrows still existed (i.e. burrow longevity) varied between burrows in the control and treatment groups. We treated the experimental group (control vs. treatment) as a fixed factor. We used the statistical software R version 3.5.1 (R Core Team 2018) for this and all subsequent statistical analysis in this paper.

### *6.3.3 Use of foreign burrows*

We conducted a second preliminary experiment to determine whether crabs would use a burrow that they did not construct. During our study, we recognized that marked individuals returned to their own burrows immediately after we had released them. We also observed that foraging individuals run back to burrows from a certain distance (i.e. up to approximately 8 -10 m) when they encounter humans. Thus, to test whether *O. quadrata* will enter any random burrow, we collected 10 individuals of various body

sizes on Waties Island (one of our pristine sites). We kept these individuals in separate plastic containers for about 10 minutes, during which time we shook the containers gently to disturb them. We then chose random burrows that were matched to the size of individual crabs and released the individuals, one by one, next to the mouths of these burrows. When an individual did not immediately try to escape or enter the burrow, we pretended to catch them to elicit a response.

#### *6.3.4 Burrow fidelity*

To understand whether the number of days during which an individual uses the same burrow (burrow fidelity) varies based on the level of human disturbances, we applied a mark and recapture technique as described above at one site for each disturbance level. For the burrow fidelity, we sampled the newly-created burrows over a one-week period in three replicate rectangular quadrats (10 m X 5 m) deployed on the seaward side of the dune vegetation at each site. We set up our quadrats near the dune vegetation because the persistence of ghost crab burrows is directly affected by the tides (Evans et al. 1976) and height on the beach (Campagnoli et al. 2018). We first measured burrow size as the largest distance across the burrow openings, which is positively related to crab size ( $r^2 = 0.98$ , Wolcott, 1978). Then, the same trapping procedure explained above was repeated every three days. During our investigation, we marked individual crabs and their burrows with the same numbers so that individual crabs and their burrows could be matched. Since some individuals destroyed their own burrows after they were disturbed, we observed the sampled burrows after a couple hours to see whether the burrows still existed. If not, we excluded that individual burrow and the crab from our data. We used a generalized linear mixed effects model (GLMM) with a Poisson

distribution to test whether burrow fidelity (number of days a burrow was used) varied among the levels of human impacts and the size of the burrow (fixed factors). To control for the latitudinal and temporal difference, sampling day (e.g. Julian days) and latitude were included as random factors in this, and all subsequent mixed models described below (Galwey et al. 2018). We then applied a Tukey's HSD test to make multiple pairwise comparisons of the burrow fidelity among the levels of human impact.

#### 6.3.5 Burrow longevity

To examine if the persistence of the ghost crab burrows themselves was influenced by the level of human impact, we observed the longevity of *O. quadrata* burrows (i.e., the number of days from creation until collapse) in three trial rectangular quadrats (20 m X 10 m) in each site listed in Table 6.1. We set up our quadrats on the seaward side of the dune vegetation. We marked the burrows in each quadrat using orange marking flags. We then marked all subsequent newly-created burrows in each site with blue or green flags for the following week. We observed the burrows marked with blue and green flags every day until they collapsed. To determine whether the burrow longevity varied among the levels of human impact or burrow size (fixed factors), we ran a generalized mixed effects model (GLMM) with a Poisson distribution, followed by a Tukey's HSD test for multiple comparisons between levels of human impacts.

Geomorphological characteristics of sandy beaches and burrow densities at the sites were determined as possible explanatory variables of the burrowing behavior (details in Supporting Information Appendix 2). We ran two generalized linear mixed effects models (GLMM) with Poisson distributions to determine whether burrow fidelity

and longevity were influenced by crab density, sand grain size and sand compaction (fixed variables).

#### *6.3.6 Energy requirement of burrowing*

We conducted an experiment to determine the energetic cost of digging for *O. quadrata*. We collected 40 individuals of a range of sizes in both sexes from Isle of Palm in September, 2017. These were transported to the University of South Carolina in Columbia, South Carolina where they were held in separate plastic containers (length 23.1 cm, width 16.2 cm, height 13.9 cm) with ~5 cm moist sand. We weighed and kept them without food for 3 days to standardize their hunger level, after which we fed them every other day with commercially purchased salmon for next 10 days. Each individual was offered 10% of its wet body weight at each feeding and uneaten food was removed after 24 hours. All of this was done in an attempt to standardize the energetic conditions across crabs.

Following this holding period, we transported the crabs back to the Isle of Palm where they were collected. We created experimental chambers by excavating 20 pits in the sand at Isle of Palm on the beach berm. This was done at night to avoid changing the temperature of sand at depth via direct sunlight. Into each pit, we placed a 121.1-liter plastic can (diameter 55.8 cm, height 81.2 cm) with approx. 50 small holes drilled into the sides and the bottom to allow moisture and temperature exchange between the inside of the chamber and the surrounding sediment. We then replaced the sand that had been removed into these experimental chambers. To mimic the sand compaction of the surrounding beach, we excavated another identical pit and measured the sand compaction

every ~20 cm during excavation using a pocket penetrometer. Then, as the chambers were filled with sand, we measured the compaction inside the chambers at each 20 cm interval and pressed the sand as necessary to achieve the same compaction level as was observed in the surrounding sediment. We surrounded the mouth of the chambers with a vertical cardboard barrier to keep the crabs from escaping and then allowed 4 hours for moisture and oxygen levels in the chambers to equilibrate. We then released an individual treatment crab onto the sand surface in each chamber and allowed them to excavate burrows. During this time, we held the other 20 control crabs in their transport containers under the same ambient environmental conditions with the treatment crabs in the field. After about 2 hours, all experimental crabs had excavated a burrow. To force the individuals to leave their burrows, we used a smoke fumigator (Pombo & Turra 2013). Twelve of the crabs exited their burrows due to the smoke, while the others remained in their burrows until we poured plaster of Paris into the burrow. We determined the burrow volume using the burrow casts created with plaster (see Supporting Information Appendix 3). We removed the second digging leg (i.e. the walking leg on the same side as the minor claw (Lucrezi & Schlacher 2014) from each treatment and control crab and immediately placed these in individually-labeled plastic bags on ice.

We used the level of glycogen content in the digging leg muscle tissue as a proxy of the energetic cost of digging. To determine the glycogen content, we used a Sigma-Aldrich Glycogen Assay Kit MAK016. We used the instructions provided by the manufacturer of the kit to perform the glycogen analysis on a subsample (~10 mg) from each leg muscle removed from the experimental and control crabs. Finally, we measured absorbance of our samples under a spectrophotometer (Biotek Synergy H1 Hybrid

Reader working with software Gen5). The glycogen content of the samples was calculated from a standard curve that we constructed simultaneously with our glycogen samples. To determine the energy level in the muscle tissues, we converted the glycogen level to energy by multiplying by 17.2 kJ g<sup>-1</sup> (Lucas 1996). Our visual inspection of graphs suggested that glycogen content in the leg tissue declined nonlinearly with burrow volume. We compared three models using AIC to determine the best model to explain this relationship (Burnham & Anderson 2002). Specifically, we fit a linear model, a negative exponential model, and a quadratic model. We also used a t test to compare the energy level in the muscle tissues between experimental crabs that burrowed and control crabs that did not.

Lastly, we estimated the annual energy demand required to support the burrowing behavior of *O. quadrata*. We did this by combining our data on burrow longevity at each of our sites and the energy expended during the removal of 1 cm<sup>3</sup> of sand (calculated from the energetic costs described above), with overall burrow volume that we have previously measured at these same sites (Gül & Griffen 2018a), and with the portion of the year on the Atlantic coast when crabs actively burrow (known to extend from April to November on the Maryland coasts, Christoffers 1986). This is admittedly a rough estimate, since it assumes that energetic costs do not change seasonally, and also does not include the costs of maintaining the burrow once it is dug. Because the burrow volume is correlated with the crab size (Chan et al. 2006), we removed the effects of crab size by regressing the log-transformed calculated burrowing energy demand data against log-transformed burrowing opening diameter (Packard & Boardman 1999). The standardized residuals obtained from this regression were used as a response variable for a 1-way

ANOVA to compare this estimated annual energy demand for burrowing in ghost crabs on beaches with different levels of human disturbances. Before this statistical analysis, we conducted a Shapiro-Wilk test of normality and Levene's test for the equality of variances to assess whether the data fit the assumptions of parametric tests. ANOVA was followed by a Tukey's HSD for multiple comparison among groups.

## **6.4 RESULTS**

### *6.4.1 Impact of marking procedure*

The trapping technique used here to determine the burrow fidelity did not alter the longevity of the burrows in the treatment group compared to the burrows in the control group under any human impact levels. The difference in burrow longevity between treatment and control groups was 0.3 day in pristine sites (GLM,  $t = -0.55$ ,  $P = 0.57$ ), 0.66 day in moderately impacted sites (GLM,  $t = -0.66$ ,  $P = 0.5$ ), 0.1 day in highly impacted sites by people (GLM,  $t = -0.77$ ,  $P = 0.81$ ), and 1 day in highly impacted sites by people and vehicles (GLM,  $t = -0.89$ ,  $P = 0.37$ ). Having a lower burrow longevity at all human disturbance levels suggested that our mark-recapture technique had a small effect, but that our sample size was too low to detect it. However, our highest sample size was 28 (range: 16-28), and given the effect size here, we would have had to investigate at least 128 burrows (Power analysis,  $\alpha = 0.05$ , power = 0.80) to detect a significant difference.

### *6.4.2 Use of foreign burrows*

We did not find any instances of a foreign crab occupying one of our focal burrows during any part of our study. This is also consistent with *O. quadrata* being

reluctant to enter burrows that were not their own in our small experiment where we tried to elicit foreign burrow use. Specifically, we observed that 9 out of 10 individuals did not enter the foreign burrow that they were released next to, even though that burrow was large enough for them. Only one individual entered shallowly into the foreign burrow, staying very close to the entrance, and when we disturbed it using a thin stick, it left the burrow immediately and ran away rather than receding further into the burrow.

#### 6.4.3 Burrow fidelity

The average burrow fidelity of *O. quadrata* was significantly lower in pristine sites (average =  $2.31 \pm 0.38$  day<sup>-1</sup>, range: 1 – 4 day<sup>-1</sup>, n = 16) compared to burrow fidelity in moderately impacted sites (average =  $5.38 \pm 0.87$  day<sup>-1</sup>, range: 1 – 10 day<sup>-1</sup>, n = 13; GLMM, Z = 5.18, P < 0.001), highly impacted sites by people (average =  $3.1 \pm 0.9$  day<sup>-1</sup>, range: 1 – 7 day<sup>-1</sup>, n = 10; GLMM, Z = 2.8, P = 0.005), and highly impacted sites by people and vehicles (average =  $2.66 \pm 0.72$  day<sup>-1</sup>, range: 1 – 7 day<sup>-1</sup>, n = 9; GLMM, Z = 2.99, P = 0.002, Fig. 6.1). We further found that the larger individuals had a stronger burrow fidelity when impact levels were pooled (GLMM, Z = 5.62, P < 0.001, Fig. 6.1). Burrow fidelity in *O. quadrata* was negatively impacted by burrow density (GLMM, Z = -4.419, P < 0.001) and sand compaction (GLMM, Z = -3.152, P = 0.0016). No impact of sand grain size on burrow fidelity was detected (GLMM, Z = -1.556, P = 0.11). No significant impact of the interaction terms was detected.

#### 6.4.4 Burrow longevity

Burrow longevity in pristine sites (average =  $2.77 \pm 0.08$  day<sup>-1</sup>, range = 1 – 6 day<sup>-1</sup>, n = 340) and in highly impacted sites by people and vehicles (average =  $3.49 \pm$



0.19 day<sup>-1</sup>, range = 1 – 8 day<sup>-1</sup>, n = 77) was similar (GLMM, Z = -0.26, P = 0.78).

Contrary to this, a greater burrow longevity was observed in moderately impacted sites

(average =  $6.71 \pm 0.26$  day<sup>-1</sup>, range = 1 – 13 day<sup>-1</sup>, n = 132; GLMM, Z = 5.74, P <

0.001) and in highly impacted sites by people (average =  $4.91 \pm 0.22$  day<sup>-1</sup>, range = 1 –

11 day<sup>-1</sup>, n = 105; GLMM, Z = 3.66, P < 0.001) compared to pristine sites (Fig. 6.2).

Also, larger burrows persisted longer than smaller ones (GLMM, Z = 11.51, P < 0.001).

There were significant interactions between burrow density and sand grain size (LM, t = -3.485, P = 0.0005), between burrow density and sand compaction rate (LM, t = -11.63, P < 0.001) and between sand compaction rate and the sand grain size (LM, t = 4.288, P < 0.001). These interactions influenced burrow longevity (Table 6.2).

#### *6.4.5 Energy requirement of burrowing*

We found that the exponential model provided the best fit to explain the relationship between glycogen content in the leg tissue and the burrow volume, but that the fit of this model was statistically indistinguishable from the fit of the quadratic model (AIC value for linear model = -129.46, AIC value for exponential model = -133.15, and AIC value for quadratic model = -132.32). The glycogen content in the digging leg tissues of the individuals in the treatment group thus declined exponentially with burrow volume (exponential model, glycogen =  $a \times e^{bx_{\text{volume}}}$ , t value for a = 3.227, P = 0.0046; t value for b = -2.827, P = 0.011; overall model P < 0.001, Fig. 6.3a). Consequently, the glycogen content in the digging leg tissues of the individuals in the treatment group declined by 51% on average compared the individuals in the control group (t test, t = 2.74, df = 37.106, P = 0.0093, Fig. 6.3b).

Ghost crabs spend 0.66 joules of energy to remove 1 cm<sup>3</sup> of sand during our experiment. Using this value as described in the Methods, we found that the calculated (conservative) annual energy demand of burrowing in ghost crabs was significantly lower for all levels of human disturbance compared to pristine sites (ANOVA,  $F = 116.8$ ,  $df = 3$ ,  $P < 0.0001$ , Fig. 6.4).

## 6.5 Discussion

We have shown that *O. quadrata* alter their burrowing behavior (e.g. burrow fidelity and longevity) under the influence of various levels of human disturbances. We have also demonstrated that *O. quadrata* likely spend a much higher amount of energy in pristine sites for their burrowing behavior compared to the sites disturbed by people. We have also shown that larger individuals generally return to the same burrow for a longer period of time compared to smaller individuals. Since burrows protect crabs from predators, cannibals, and environmental influences such as strong winds, cold, and hot weather (Christoffers 1986, Lucrezi & Schlacher 2014) and desiccation (Antia 1989, Gül & Griffen 2018a), changing the energetics of this behavior is likely to shift the ecology, physiology, and demographics of *O. quadrata*. These results have important implications for this system and for our understanding of ecological changes due to human disturbance more broadly.

### 6.5.1 Implications for this system

Burrow fidelity and longevity increased at sites with moderate human disturbance compared to heavily disturbed and pristine sites. Specifically, individuals used the same burrows for up to 10 days at sites with moderate human disturbance. Pristine sites on the

coast of South Carolina have the highest ghost crab abundance and the lowest sand compaction rates (Gül & Griffen 2018a, 2018b; Table B.2, Supporting information). However, while an inverse relationship between population density and the duration of burrow use has been reported (Hughes 1966), the sand compaction rate may be the main mechanism that determines the length of the burrow persistence, as suggested by the positive relationship between sand compaction and burrow longevity reported here.

The positive relationship between the size of the individuals and the length of time during which a burrow is used likely reflects the location of burrows of different sizes on the shore. Larger burrows are found much closer to the back shore on South Carolina beaches, while smaller crabs are outcompeted on the upper shore and are relegated to digging their burrows lower down on the shore (Gül & Griffen 2018b). An inverse correlation between tidal height and burrow persistence exists (Hughes, 1966, Evans et al. 1976, Costa et al. 2018), likely due simply to increased likelihood of inundation by waves lower down on the shore. We attempted to control for effects of tidal influence by examining only burrows at the same relative tidal height at all sites; however, our transects were wide enough (e.g. 10 m) to contain small burrows at the lower edges of the transects. Alternatively, the visual cues such as mounds and debris, which are used by *O. quadrata* to recognize their burrows (Hughes 1966, Lucrezi & Schlacher 2014, Costa et al. 2018), may be removed more frequently around the smaller burrows due to their relatively lower location on the shore.

We found that the glycogen level in the leg tissues dropped by almost 50% after 2 hours of burrowing. Assuming that this energy required to initially dig a burrow is greater than the energy required to maintain a burrow, the longevity of burrow use and

the energy allocated for digging annually should be inversely related to each other, and this relationship is influenced by human disturbance in *O. quadrata* populations. Specifically, we calculated that ghost crab living in pristine sites have the highest burrowing energy demand, while individuals living under human disturbance decrease the amount of annual energy requirements for burrowing by creating smaller and simpler burrows (Lucrezi & Schlacher 2010, Schlacher & Lucrezi 2010, Gül & Griffen 2018) and by increasing their burrow fidelity and the longevity. These reductions in energy allocation to burrowing may be required if *O. quadrata* gain less energy through foraging on beaches that are more highly disturbed, as human activities also negatively impact common ghost crab prey such as bean clams (*Donax* spp.), mole crabs (*Emerita* spp.) and sandy beach coleoptera (*Phaleria* spp.) (Schlacher et al. 2008, Sheppard et al. 2009, González et al. 2014, Cardoso et al. 2016).

Finally, we examined only the amount of energy that is allocated by individuals based on their initial burrowing behaviors. However, crabs will also need to expend energy to maintain their burrows, and these daily maintenance costs should also differ across beaches based on human impacts because of the influence of humans on sand compaction. However, our findings that burrow longevity only slightly exceed burrow fidelity (i.e., burrows collapse after they are left vacant for ~1 day) suggests that burrow maintenance is considerable. Further research is needed to examine the relative costs of initial burrow creation and burrow maintenance.

### *6.5.2 Broader implications for ecology*

Our study has at least two broader implications for the population ecology of species used as bioindicators of human disturbances. First, in agreement with countless other studies, our study shows that species alter their behaviors and daily activities, and therefore their energetic balance, in the presence of human disturbance. Despite the fact that lower population density and smaller individual sizes are widely used as common responses of bioindicator species to human disturbances (Carignan & Villard 2002, Spellerberg 2005, Heink & Kowarik 2010, Siddig et al. 2016), the mechanistic reasons for those demographic changes are often not well understood. Whereas, many studies have examined the behavioral and energetic impacts of indirect human disturbances including climate change and contamination (Williams et al. 2006, Bonnard et al. 2009, Griffen 2017, Schmidt et al. 2017), relatively few studies have focused on the energetic consequences of direct human impacts (e.g. tourism, coastal reclamation etc.) on bioindicator species (but see Toro et al. 2003, Adams & Ham 2011). Our results highlight that focusing on behavioral changes and their energetic consequences may elucidate the mechanistic reasons behind the declines in individual sizes, because species may exhibit tradeoffs between their growth rate and daily activities that are crucial for their survival.

Second, the link between the levels of human disturbance and energy allocation suggests that this mechanistic link may provide improved power to predict the impacts of future human disturbances. While examining changes in abundance and individual body sizes within populations is a quick and cost-effective technique for assessing the extent of human disturbance (Carignan & Villard 2002, Spellerberg 2005, Cortes et al. 2013), documenting these demographic changes does not provide any understanding regarding

the mechanism(s) behind these responses. To go beyond documenting existing trends and to make predictions about responses to future conditions, ecologists need mechanistic approaches that are applicable to various species under a variety of disturbance types and in different systems. This is especially urgent given the increasing frequency and strength of anthropogenic disturbance as human population sizes increase (Vitousek et al. 1997, Davenport & Davenport 2006, Halpern et al. 2008). Previous work has argued that understanding the physiological and energetic state of organisms can provide a level of mechanistic predictive power for forecasting future conditions (Pörtner & Farrell 2008). Our study supports this idea and demonstrates how a bioenergetics approach may be used to explore the consequences of behavioral changes that accompany habitat disturbance by humans.

## **6.6 Acknowledgement**

We thank Esra Erdil Gül for assistance in field observations on burrow longevity, and Ahmet Postağasi for his assistance in burrowing energy requirement experiment in the field. We thank Zachary Cannizzo and Crystal Conaway for their assistance in glycogen analysis. We thank the Baruch Institute, Coastal Carolina University, and DeBordieu Colony management for permission to access their facilities. This research was funded by the Ministry of National Education, Republic of Turkey (awarded to MRG).

## 6.7 Tables

Table 6.1. Levels of the urbanization indicators from absent (0) to extremely high (5) level and the urbanization index (UI) of the study sites. The sites were aligned based on their latitudes from north to south. (A=proximity to urban centers, B = buildings on the sand and dunes, C= beach cleaning, D= number of vehicles on the sand, E= frequency of visitors, F= infrastructures).

Site	A	B	C	D	E	F	UI
Waties Island 1	0	0	0	0	1	0	0.03
Waties Island 2	0	0	0	0	1	0	0.03
Waties Island 3	0	0	0	0	1	0	0.03
N. Myrtle Beach 1	5	4	5	4	5	5	0.93
N. Myrtle Beach 2	5	4	5	4	5	5	0.93
Myrtle Beach 1	5	4	5	5	5	5	0.96
Myrtle Beach 2	5	4	5	5	5	5	0.96
Garden City Beach	5	5	5	3	5	4	0.9
Pawley's Island 1	3	4	0	0	2	2	0.36
Pawley's Island 2	3	4	0	0	2	2	0.36
Debidue Island 1	1	0	0	0	1	0	0.06
Debidue Island 2	1	0	0	0	1	0	0.06
Isle of Palm 2-1	5	4	3	2	4	3	0.7
Isle of Palm 2-2	5	4	3	2	4	3	0.7
Isle of Palm 1-1	3	3	0	0	2	1	0.3
Isle of Palm 1-2	3	3	0	0	2	1	0.3
Sullivan's Island 1	4	3	3	3	4	3	0.66
Sullivan's Island 2	4	3	3	3	4	3	0.66
Folly Beach	4	4	1	0	3	3	0.5
Burkes Beach	4	2	2	2	5	5	0.66

Table 6.2. Results of generalized linear mixed model (GLMM) testing the effects of fixed factors on the burrow longevity of *O. quadrata*. Latitudes of the study sites and sampling days (as Julian days) were included as random effects.

<b>Fixed factors</b>	<b>S.E.</b>	<b>Z-value</b>	<b>P - value</b>
<i>A</i> (burrow density)	1.305	-10.305	<0.001*
<i>B</i> (grain size)	3.331	-6.15	<0.001*
<i>C</i> (sand compaction)	7.294	-7.07	<0.001*
<i>A x B</i>	4.325	9.076	<0.001*
<i>B x C</i>	14.262	10.046	<0.001
<i>A x B x C</i>	49.093	-8.331	<0.001*
<b>Random factors</b>	<b>Variance component</b>	<b>S.E.</b>	
<i>Latitude</i>	<0.001	0.00014	
<i>Julian days</i>	<0.001	0.1412	



## 6.8 Figures

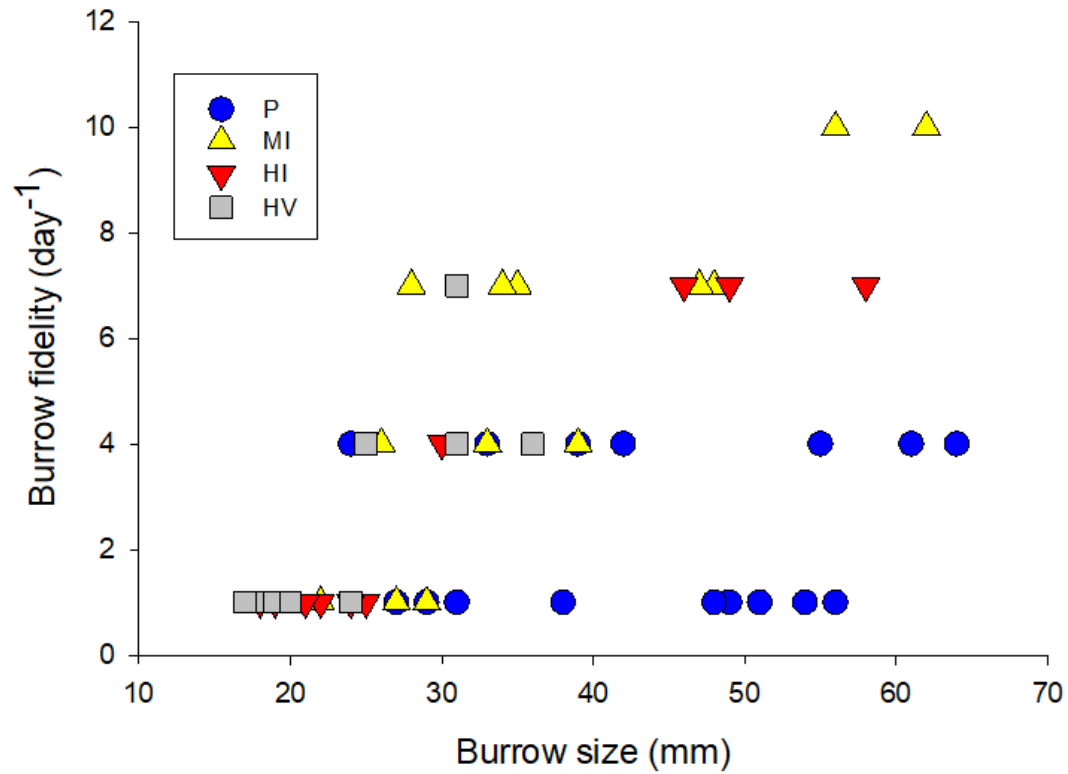


Figure 6.1. The relationship between the burrow fidelity and the burrow opening diameter at the sites with various levels of human disturbance. Burrow fidelities were observed in Waties Island (pristine site), Pawley's Island (moderately impacted site), Isle of Palm (highly impacted site by people) and Garden City Beach (highly impacted site by people and vehicles).

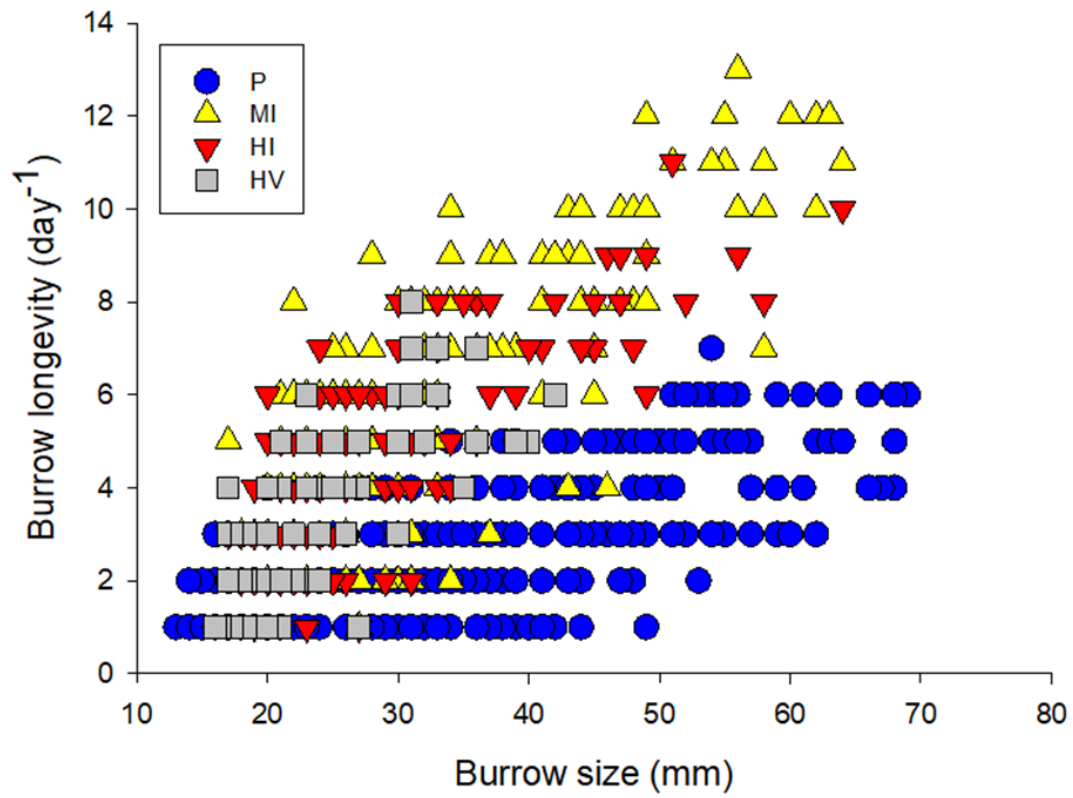


Figure 6.2. The relationship between the burrow longevity and the burrow opening diameter at sites with various levels of human disturbance. Burrow longevity was observed daily at all sites. Study sites the same as listed in Table 1.

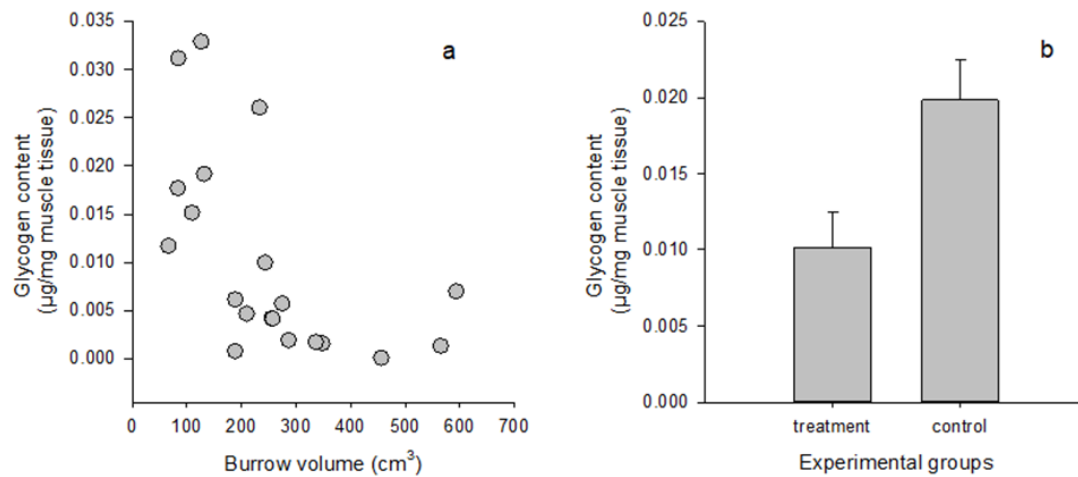


Figure 6.3. The relationship between the burrow volume and the glycogen content in the second digging leg tissue of crabs ( $n = 20$ ), *O. quadrata* (a). Variation in the glycogen content in the second digging leg tissue of the treatment and the experiment crabs (b). Burrowing experiment was conducted on Isle of Palm in 2017.

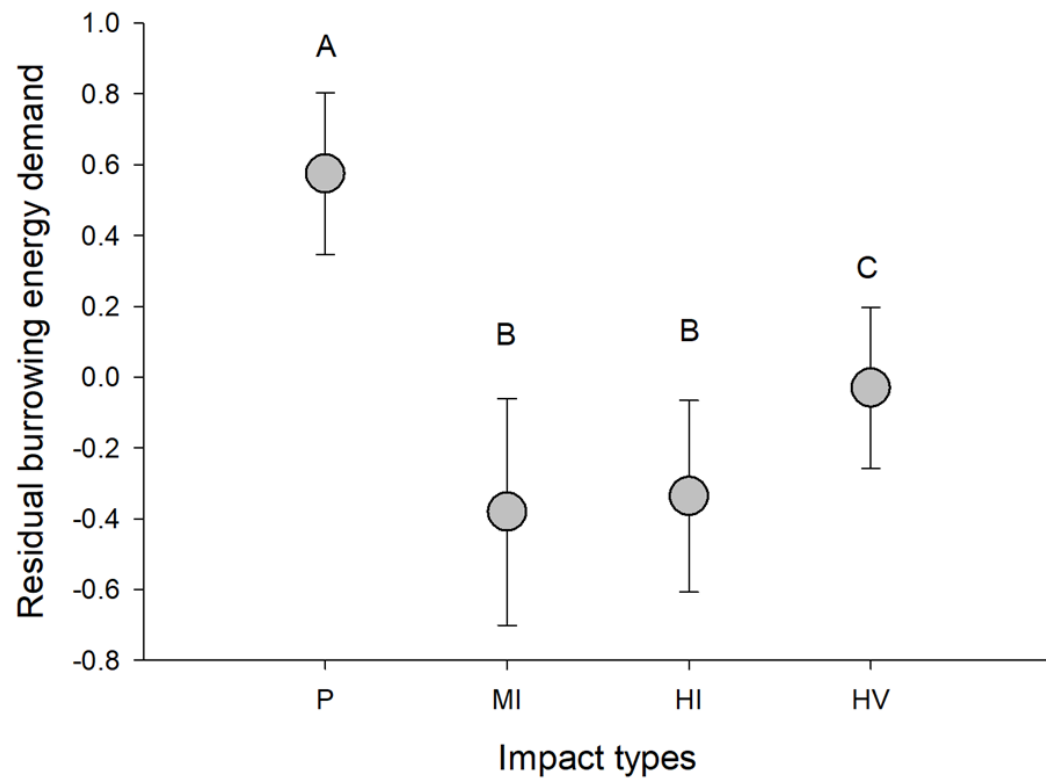


Figure 6.4. Variation in residual calculated annual burrowing energy demand (mean  $\pm$  S.D.) of *O. quadrata* under various levels of human disturbance. Letters within graph represent significant differences.

## 6.9 Literature Cited

- Adams, S. M., & Ham, K. D. (2011). Application of biochemical and physiological indicators for assessing recovery of fish populations in a disturbed stream. *Environmental Management*, 47(6), 1047- 1063. <https://doi.org/10.1007/s00267-010-9599-7>
- Aheto, D., Asare, C., Mensah, E., & Aggrey-Fynn, J. (2011). Rapid assessment of anthropogenic impacts on exposed sandy beaches in Ghana using ghost crabs (*Ocypode* spp.) as ecological indicators. *Momona Ethiopian Journal of Science*, 3(2), 93–103.
- Antia, E.E. (1989). Beach cusps and burrowing activity of crabs on a fine-grained sandy beach, southeastern Nigeria. *Journal of Coastal Research*, 5, 263–270.
- Barros, F. (2001). Ghost crabs as a tool for rapid assessment of human impacts on exposed sandy beaches. *Biological Conservation*, 97, 399–404.
- Bartolini, F., Penha-Lopes, G., Limbu, S., Paula, J., & Cannicci, S., (2009). Behavioural responses of mangrove fiddler crabs (*Uca* spp.) to urban sewage loadings: results of a mesocosm approach. *Marine Pollution Bulletin*, 58, 1860- 1867.  
[doi:10.1016/j.marpolbul.2009.07.019](https://doi.org/10.1016/j.marpolbul.2009.07.019)
- Bonnard, M., Romeo, M., & Amiard-Triquet, C. (2009). Effects of copper on the burrowing behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and the bivalve *Scrobicularia plana*. *Human and Ecological Risk Assessment*, 15, 11–26. <https://doi.org/10.1080/10807030802614934>
- Burnham, K.P., & Anderson, D.R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer-Verlag.

- Campagnoli, M.L., Pombo, M., & Turra, A. (2018). Ghost crab burrows simulation shows differential across-shore persistence. *Crustaceana*, 91(7), 821- 830.  
<https://doi.org/10.1163/15685403-00003802>
- Cardoso, R. S., Barboza, C. A. M., Skinner, V. B., & Cabrini, T. M. B. (2016). Crustaceans as ecological indicators of metropolitan sandy beach health. *Ecological Indicators*, 62, 154–162. <https://doi.org/10.1016/j.ecolind.2015.11.039>
- Carignan, V., & Villard, M.A. (2002). Selecting indicator species to monitor ecological integrity: a review. *Environmental Monitoring and Assessment*, 78(1), 45- 61. PMID: 12197640
- Carr, M.H., Neigel, J.E., Estes, J.A., Andelman, S., Warner, R.R. & Largier, J.L. (2003). Comparing marine and terrestrial ecosystems: implications for the design of coastal marine reserves. *Ecological Applications*, 13, S90– S107.
- Chandurvelan, R., Marsden, I. D., Glover, C. N., & Gaw, S. (2015). Assessment of a mussel as a metal bioindicator of coastal contamination: Relationships between metal bioaccumulation and multiple biomarker responses. *The Science of the Total Environment*, 511, 663- 675. <http://dx.doi.org/10.1016/j.scitotenv.2014.12.064>
- Chan, B.K.K., Chan, K.K.Y., & Leung, P.C.M. (2006). Burrow architecture of ghost crab *Ocypode ceratophthalma* on a sandy shore in Hong Kong. *Hydrobiologia*, 560(1): 43–49.  
<https://doi.org/10.1007/s10750-005-1088-2>
- Christoffers, E. W. (1986). Ecology of the ghost crab *Ocypode quadrata* (Fabricius) on Assateague Island, Maryland and the impacts of various human uses of the beach on their

distribution and abundance. PhD thesis, Michigan State University, East Lansing, MI, USA.

Cortes, R.M.V., Hughes, S.J., Pereira, V.R., & Varandas, S.D.P. (2013). Tools for bioindicator assessment in rivers: the importance of spatial scale, land use patterns and biotic integration. *Ecological Indicators*, 34, 460- 477.

<https://doi.org/10.1016/j.ecolind.2013.06.004>

Costa, L. L., Madureira, J. F., & Zalmon, I. R. (2018). Changes in the behaviour of *Ocypode quadrata* (Fabricius, 1787) after experimental trampling. *Journal of Marine Biological Association of the United Kingdom*, 1- 6.

<https://doi.org/10.1017/S0025315418001030>

Costa, L., Tavares, D. C., Suciú, M. C., & Rangel, D. (2017). Human-induced changes in the trophic functioning of sandy beaches. *Ecological Indicators*, 82, 304- 315. DOI: 10.1016/j.ecolind.2017.07.016

Culbertson, J. B., Valiela, I., Peacock, E. E., Reddy, C. M., Carter, A., & Vanderkruik, R. (2007). Long-term biological effects of petroleum residues on fiddler crabs in salt marshes. *Marine Pollution Bulletin*, 54(7), 955- 962.

<https://doi.org/10.1016/j.marpolbul.2007.02.015>

Davenport, J., & Davenport, J. L. (2006). The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine, Coastal and Shelf Science*, 67(1-2), 280- 292. <https://doi.org/10.1016/j.ecss.2005.11.026>

- Defeo, O., McLachlan, A., Schoeman, D. S., Schlacher, T. A., Duagm J., Jones, A., ...Scapini, F. (2009). Threats to sandy beach ecosystems: A review. *Estuarine, Coastal and Shelf Science*, 81(1), 1- 12. <https://doi.org/10.1016/j.ecss.2008.09.022>
- Erdmann, M. V., & Caldwell, R. L. (1997). Stomatopod crustaceans as bioindicators of marine pollution stress on coral reefs. *Proceedings of the Eighth International Coral Reef Symposium*, 2, 1165- 1170.
- Evans, S.M., Cram, A., Eaton, K., Torrance, R. & Wood, V. (1976). Foraging and agonistic behaviour in the ghost crab *Ocypode kuhlii* de Haan. *Marine and Freshwater Behaviour and Physiology*, 4, 121–135.
- Friend, G. R. (1993). Impact of fire on small vertebrates in mallee woodlands and heathlands of temperate Australia: a review. *Biological Conservation*, 65, 99- 114.
- Fox, J., & Weisberg, S. (2018). *An R Companion to Applied Regression* (3rd edition). SAGE: Thousand Oaks, CA, USA.
- Galwey, N.W. (2014). *Introduction to Mixed Modelling: Beyond Regression and Analysis Of Variance* (2nd edition). Wiley, Chichester.
- González, S. A., Yáñez-Navea, K., & Muñoz, M. (2014). Effect of coastal urbanization on sandy beach coleoptera *Phaleria maculate* (Kulzer, 1959) in northern Chile. *Marine Pollution Bulletin*, 83(1): 265–274. <https://doi.org/10.1016/j.marpolbul.2014.03.042>
- Gray, M. A., Baldauf, S. L., Mayhew, P. J., & Hill, J. K. (2007). The response of avian feeding guilds to tropical forest disturbance. *Conservation Biology*, 21(1), 133- 141. DOI: 10.1111/j.1523-1739.2006.00557.x



- Griffen, B. D. (2018). Modeling the metabolic costs of swimming in polar bears (*Ursus maritimus*). *Polar Biology*, 41(3), 491- 503. <https://doi.org/10.1007/s00300-017-2209-x>
- Griffiths, J. R., Kadin, M., Nascimento, F. J. A., Tamelander, T., Törnroos, A., Bonaglia, S.,.....Winder, M. (2017). The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. *Global Change Biology*, 23, 2179- 2196. doi: 10.1111/gcb.13642
- Gül, M. R., & Griffen, B. D. (2018a). Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores. *PLoS ONE* 13(12): e0209977. <https://doi.org/10.1371/journal.pone.0209977>
- Gül, M. R., & Griffen, B. D. (2018b). A reliable bioindicator of anthropogenic impact on the coast of South Carolina. *Southeastern Naturalist*. 2018; 17(2): 357–364. <https://doi.org/10.1656/058.017.0217>
- Hallock, P., Lidz, B. H., Cockey-Burkhard, E. M., & Donnelly, K. B. (2003). Foraminifera as bioindicators in coral reef assessment and monitoring: The FORAM Index. *Environmental Monitoring and Assessment*, 81, 221–238.
- Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D’Agrosa, C., .....Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, 319, 948- 952.
- Heink, U., Kowarik, I., 2010. What are indicators? On the definition of indicators in ecology and environmental planning. *Ecological Indicators*, 10 (3), 584–593. doi:10.1016/j.ecolind.2009.09.009

- Hobbs, C.H., Landry, C.B. & Perry, J.E. 2008. Assessing anthropogenic and natural impacts on ghost crabs (*Ocypode quadrata*) at Cape Hatteras National Seashore, North Carolina. *Journal of Coastal Research*, 24, 1450–1458. <https://doi.org/10.2112/07-0856.1>
- Hughes, D. A. (1966). Behavioural and ecological investigations of the crab *Ocypode ceratophthalmus* (Crustacea: Ocypodidae). *Journal of Zoology*, 150, 129–143.
- Hunter, R. D., & Elder, H.Y. (1989). Burrowing dynamics and energy cost of transport in the soft-bodied marine invertebrates *Polyphysia crassa* and *Priapulius caudatus*. *Journal of Zoology*, 218(2), 209- 222.
- Jokimäki, J., Suhonen, J., Jokimäki-Kaisanlahti, M. L., & Carbó-Ramírez, P. (2016). Effects of urbanization on breeding birds in European towns: Impacts of species traits. *Urban Ecosystems*, 19(4), 1565- 1577. doi: 10.1007/s11252-014-0423-7
- Jonah, F.E., Agbo, N.W., Agbeti, W., Adjei-Boateng, D., & Shimba, M.J. (2015). The ecological effects of beach sand mining in Ghana using ghost crabs (*Ocypode* species) as biological indicators. *Ocean Coastal Management*, 112, 18- 24.  
<https://doi.org/10.1016/j.ocecoaman.2015.05.001>
- Leo, E., Dahlke, F. T., Storch, D., Pörtner, H. O., & Mark, F. C. (2018). Impact of ocean acidification and warming on the bioenergetics of developing eggs of Atlantic herring *Clupea harengus*. *Conservation Physiology*, 6(1), coy050.  
<https://doi.org/10.1093/conphys/coy050>
- Lucas, A. (1996). *Bioenergetics of Aquatic Animals*. Taylor & Francis, London.

Lucrezi, S., & Schlacher, T. A. (2010). Impacts of off-road vehicles (ORVs) on burrow architecture of ghost crabs (Genus *Ocypode*) on sandy beaches. *Environmental Management*, 45(6), 1352- 1362. DOI 10.1007/s00267-010-9491-5

Lucrezi, S. & Schlacher, T. A. (2014). The ecology of ghost crabs-a review. *Oceanography and Marine Biology- Annual Review*, 52, 201- 256.

Lucrezi S, Schlacher TA, Robinson W. (2009). Human disturbance as a cause of bias in ecological indicators for sandy beaches: experimental evidence for the effects of human trampling on ghost crabs (*Ocypode* spp.). *Ecological Indicators*, 9 (5): 913–921.

<https://doi.org/10.1016/j.ecolind.2008.10.013>

Neves, F. M., & Bemvenuti, C. E. (2006). The ghost crab *Ocypode quadrata* (Fabricius, 1787) as a potential indicator of anthropic impact along the Rio Grande do Sul coast, Brazil. *Biological Conservation* 133, 431–435.

<https://doi.org/10.1016/j.biocon.2006.04.041>

Nomura, F., Rossa-Feres, C., & Langeani, F. (2009). Burrowing behavior of *Dermatonotus muelleri* (Anura, Microhylidae) with reference to the origin of the burrowing behavior of Anura. *Journal of Ethology*, 27, 195- 201.

Packard, G.C., & Boardman, T.J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comparative Biochemistry and Physiology B*, 122, 37- 44.

- Pearce, J. L. & Venier, L. A. (2006). The use of ground beetles (Coleoptera: Carabidae) and spiders (Araneae) as bioindicators of sustainable forest management: A review. *Ecological Indicators*, 6(4), 780- 793. doi.org/10.1016/j.ecolind.2005.03.005
- Pombo, M., & Turra, A. (2013). Issues to be considered in counting burrows as a measure of Atlantic ghost crab populations, an important bioindicator of sandy beaches. *PLoS ONE*, 8(12): e83792. https://doi.org/10.1371/journal.pone.0083792
- Pörtner, H. O., & Farrell, A.P. (2008). Physiology and climate change. *Science*, 322, 690-692. DOI: 10.1126/science.1163156
- Rainio, J., & Niemelä, J. (2003). Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity & Conservation*, 12, 487- 506.
- Ribeiro, P. D., Christy, J. H., Rissanen, R. J. & Kim, T. W. (2006). Males are attracted by their own courtship signals. *Behavioral Ecology and Sociobiology*, 61, 81- 89.
- Schlacher, T. A., & Lucrezi, S. (2010). Experimental evidence that vehicle traffic changes burrow architecture and reduces population density of ghost crabs on sandy beaches. *Vie Milieu- Life Environment*, 60, 313- 320.
- Schlacher, T. A., Lucrezi, S., Connolly, R. M., Peterson, C. H., Gilby, B. L., Maslo, B., ..... Schoeman, D. S. (2016a). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal and Shelf Science*, 169, 56- 73. https://doi.org/10.1016/j.ecss.2015.11.025

Schlacher, T.A., Thompson, L.M.C., & Price, S. (2007). Vehicles versus conservation of invertebrates on sandy beaches: quantifying direct mortalities inflicted by off-road vehicles (ORVs) on ghost crabs. *Marine Ecology*, 28, 354–367.

Schlacher, T.A., Thompson, L.M.C., & Walker, S.J. (2008). Mortalities caused by off-road vehicles (ORVs) to a key member of sandy beach assemblages, the surf clam *Donax deltoides*. *Hydrobiology* 610, 345–350.

Schmidt, M., Gerlach, G., Leo, E., Kunz, K.L., Swoboda, S., Pörtner, H.O.,....Storch, D. (2017). Impact of ocean warming and acidification on the behaviour of two co-occurring gadid species, *Boreogadus saida* and *Gadus morhua*, from Svalbard. *Marine Ecology Progress Series*, 571, 183- 191. <https://doi.org/10.3354/meps12130>

Siddig, A.A.H., Ellison, A.M., Ochs, A., Villar-Leeman, C., & Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in ecological indicators. *Ecological Indicators*, 60, 223-230.  
[doi:10.1016/j.ecolind.2015.06.036](https://doi.org/10.1016/j.ecolind.2015.06.036)

Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. *Animal Behaviour*, 85(5), 1077- 1088.  
<https://doi.org/10.1016/j.anbehav.2013.02.017>

Sheppard, N., Pitt, K. A., & Schlacher, T. A. (2009). Sub-lethal effects of off-road vehicles (ORVs) on surf clams on sandy beaches. *Journal of Experimental Marine Biology and Ecology*, 380, 113–118. <https://doi.org/10.1016/j.jembe.2009.09.009>

Spellerberg, I. (2005). *Monitoring ecological change* (2nd ed.). Cambridge, UK: Cambridge University Press.

Steiner, A.J. & Leatherman, S.P. (1981). Recreational impacts on the distribution of ghost crab *Ocypode quadrata* Fab. *Biological Conservation*, 20, 111–122.

Stelling-Wood, T.P., Clark, G.F., & Poore, A.G.B. (2016). Responses of ghost crabs to habitat modification of urban sandy beaches. *Marine Environmental Research*, 116, 32–40. <https://doi.org/10.1016/j.marenvres.2016.02.009>

Thomas, Y., Pouvreau, S., Alunno-Bruscia, M., Barillé, L., Bryère, P., & Gernez, P. (2016). Global change and climate-driven invasion of the Pacific oyster (*Crassostrea gigas*) along European coasts: a bioenergetics modelling approach. *Journal of Biogeography*, 43(3), 568- 579. <https://doi.org/10.1111/jbi.12665>

Toro, B., Navarro, J. M., & Palma-Fleming, H. (2003). Relationship between bioenergetics responses and organic pollutants in the giant mussel, *Choromytilus chorus* (Mollusca: Mytilidae). *Aquatic Toxicology*, 63(3), 257- 269. [https://doi.org/10.1016/S0166-445X\(02\)00181-9](https://doi.org/10.1016/S0166-445X(02)00181-9)

Villegas-Amtmann, S., Schwarz, L. K., Sumich, J. L., & Costa, D. P. (2015). A bioenergetics model to evaluate demographic consequences of disturbance in marine mammals applied to gray whales. *Ecosphere*, 6(10), art183

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of the Earth's ecosystems. *Science*, 277, 494–499.

- Wildsmith, M.D., Rose, T. H., Potter, I. C., Warwick, R. M., Clarke, K. R., & Valesini, F. J. (2009). Changes in the benthic macroinvertebrate fauna of a large microtidal estuary following extreme modifications aimed at reducing eutrophication. *Marine Pollution Bulletin*, 58(9), 1250- 1262. doi: 10.1016/j.marpolbul.2009.06.008
- Williams, R., Lusseau, D., & Hammond, P. S. (2006). Estimating relative energetic costs of human disturbance to killer whales (*Orcinus orca*). *Biological Conservation*, 133(3), 301- 311. <https://doi.org/10.1016/j.biocon.2006.06.010>
- Wolcott, T.G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fab.) on an ocean beach: scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31, 67–82.
- Wolcott, T. G., & Wolcott, D. L. (1984). Impact of off-road vehicles on macroinvertebrates of a mid-atlantic beach. *Biological Conservation*, 29(3), 217- 240.
- Wong, B. B. M., & Candolin, U. (2015). Behavioral responses to changing environments. *Behavioral Ecology*, 26(3), 665- 673. doi:10.1093/beheco/aru183

CHAPTER 7

HUMAN IMPACTS CHANGE DIET, ENERGY STORAGE, AND  
REPRODUCTION IN BIOINDICATOR SPECIES<sup>1</sup>

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<sup>1</sup>Gül MR & Griffen BD. Human impacts change diet, energy storage, and reproduction in bioindicator species. To be submitted to *Journal of Animal Ecology*.



## **7.1. Abstract**

Demographic shifts in populations of certain species (e.g. bioindicators) are widely examined as a response to the human disturbances that often shape ecosystems, globally. Yet, the mechanistic drivers of these demographic changes are often poorly understood. The Atlantic ghost crab, *Ocypode quadrata*, as a widely used bioindicator species of human disturbances on sandy shores, experiences similar demographic changes as other bioindicator species by reducing their population abundance and individual body sizes. Previous studies have focused on the direct influences of human disturbances such as variations in mortality rates and burrowing characteristics in order to explain observed demographic changes in this species. Here, we examine the potential for indirect influences of human disturbance to mechanistically cause observed demographic responses of this bioindicator. Specifically, we investigate whether variation in habitat quality associated with the strength of human disturbance results in reduced diet quality and therefore reduced physiological condition of ghost crabs. We demonstrate an inverse relationship between diet quality and the strength of human disturbance, with resulting reductions in the physiological and reproductive condition of ghost crabs with human disturbance. Our study shows that indirect human disturbance can explain existing variation in demographic characteristics of this bioindicator species, and highlights the importance of understanding bioenergetic relationships between species habitat use and human impacts.

## **7.2 Introduction**

Human beings influence ecosystems in a variety of ways, from tourism to overfishing and pollution (Vitousek et al. 1997). Coastal regions have experienced the

strongest impacts, due to their attractiveness because of the ecosystem services that they offer to residents and nonresidents alike (Davenport & Davenport 2006, Halpern et al. 2008). Heavy human influence in coastal areas has led to the decline of coastal species diversity and abundance (Schooler et al. 2019) and to shifts in interspecific interactions (Maxwell et al. 2013). As the human population continues to increase, coastal regions and species inhabiting those areas will experience ever stronger human pressure (Vitousek et al. 1997, Halpern et al. 2008). Consequently, there is an urgent need for accurate and realistic predictions about the responses of species living in coastal regions to these increasing disturbances in order to develop efficient conservation and management plans.

The most obvious influence of human disturbance in coastal regions is the reduced abundance and reduced size/age distributions of populations. These demographic changes are easily observable in certain species that have become bioindicator species of human impacts (Cortes et al. 2013, Siddig et al. 2016). These easily observable changes to population abundance and size structure in response to human disturbances are likely the result of changes in the fundamental biological processes and features of these organisms. For instance, pollution, toxicity, and climate change can alter the protein content, internal cell structures, and heart rate in limpets (reviewed in Reguera et al. 2018), and mussels (reviewed in Azizi et al. 2018). Similarly, cardiac and respiratory rates of fish and crayfish are altered in low water quality (reviewed in Kuklina et al. 2013).

Changes in abundance and size structure of populations may also functionally stem from changes in the behavior of organisms, such as changes to feeding habits

(Jokimäki et al. 2016; Griffiths et al. 2017) and trophic interactions (Kiskaddon et al. 2019). For instance, reduced habitat quality because of human disturbance can reduce individual growth (Feary et al. 2009), cause deteriorated physiological state (Griffen & Norelli 2015), alter behavior (Rasmussen & Belk 2012, Belgrad et al. 2017), and reduce fitness (Franklin et al. 2000, Griffen 2014), ultimately altering population demographics (Vasconcelos et al. 2014) and intra-specific interactions (Fox 1975, Wise 2006).

Finally, habitat quality may also determine the source and sink dynamics of populations (Pulliam 1988) by altering growth and survival rates (Seward et al. 2018). In such cases, populations in lower quality habitats have low intrinsic growth rates (i.e., sinks) and only persist because of recruitment derived from surrounding higher habitat-quality sites (i.e., sources) (Pulliam 1988, Lyon et al. 2019). Moreover, a source might turn into a sink or vice versa when the conditions change (reviewed in Loreau et al. 2013). Therefore, understanding the influences of human disturbance on habitat quality and the subsequent impacts on the condition and success of individual animals is important for understanding and predicting the trajectory of populations in coastal regions that are heavily influenced by people.

Here, we investigate how the strength of human disturbance influences habitat quality (i.e., food availability), diet choice, and subsequent energy storage and reproductive effort in a widespread bioindicator species, the Atlantic ghost crab, *Ocypode quadrata*. The Atlantic ghost crab is a semi-terrestrial species that feeds on a variety of prey items from dune plants to swash zone organisms, as well as cannibalizing conspecifics (Wolcott 1978, Lucrezi & Schlacher 2014, Morrow et al. 2014, Tewfik et al. 2016). Their preferred prey includes mole crabs, *Emerita talpoida*, and coquina clams,

*Donax variabilis*, likely due to the high caloric content of these prey (Wolcott 1978). However, ghost crab diets change based on prey availability (Morrow et al. 2014, Tewfik et al. 2016). In addition to predatory impacts, ghost crabs form an important link in sandy shore food webs as they are consumed by higher predators, such as raccoons (Fisher and Tevesz 1979;). Ghost crabs are widely used as a bioindicator species for detecting human disturbance on sandy shores (Schlacher et al. 2016 and citations therein, Gül & Griffen 2018a, 2018b) given that they display the characteristic decline in population abundance and individual body sizes on sandy beaches with human disturbance (Schlacher et al. 2016, Gül & Griffen 2018a, 2018b). Previous studies document reduced potential prey items for ghost crab at disturbed sites (Defeo & de Alava 1995, Schlacher et al. 2008, Sheppard et al. 2009, Cardoso et al. 2016), suggesting a bottom-up effects on sandy shores. Thus, here we predicted that ghost crab will qualitatively and quantitatively shift their diet at disturbed sites compared to more pristine sites (Lucrezi & Schlacher 2014, Morrow et al. 2014, Tewfik et al. 2016). We further predicted that this diet shift would result in deteriorated physiological condition at disturbed sites via lower energy stores and reduced reproductive effort.

## **7.3 Materials and Methods**

### *7.3.1 Study sites*

We investigated the link between habitat quality and the diet and physiological condition of the Atlantic ghost crab, *O. quadrata* on 20 South Carolina sandy beaches that differ in the strength of human disturbance (Table C.1, Supporting information). We categorized the level of disturbance at each of our study sites using an urbanization index (UI) as described in González et al. (2014) that integrated six different variables that

represent the levels of urbanization of the beaches during the summer of 2016 and 2017. Specifically, we collected data on proximity of each site to major cities, the presence of buildings in the area of each study site, number of visitors and vehicles on the sand at each site, frequency of cleaning, and the presence of amenities. The UI values across our study sites ranged from 0.03 to 0.96. We grouped our study sites as pristine sites (P: UI values lower than 0.25), moderately impacted sites (MI: UI values between 0.26 and 0.50), highly impacted sites by people (HI: UI values between 0.51 and 0.75), and highly impacted sites by people and vehicles (HV: UI values higher than 0.76). For a more detailed description of the study sites please see Gül and Griffen (in review (a)).

### *7.3.2 Ghost crab collection*

We collected a total of 316 ghost crabs (154 individuals in 2016 and 162 individuals in 2017) of both sexes from sandy beaches with different levels of human disturbance using a dip net. We collected 4 to 12 crabs of each sex from each site (Table C.1, Supporting information). We collected crabs over a wide range of sizes (14 – 50.5 mm CW) because ghost crabs can have ontogenetic diet shifts (Lim et al. 2016) and we wanted to get an overall holistic view of the population diet at each site. We collected all crabs just before sunrise to increase the likelihood that these nocturnal foragers would have full stomachs. All collected individuals were immediately euthanized in the field by placing them on ice.

### *7.3.3 Habitat quality*

We examined the density of potential prey items as a proxy of habitat quality for ghost crabs in 2017. We therefore assume that food availability across sites was relatively similar between 2016 and 2017. We specifically investigated the most common prey

items of ghost crabs which are mole crabs (*Emerita talpoida*), coquina clams (*Donax variabilis*), carrion that was drifted onto the shore by the tides (e.g. fish, jelly fish and horse shoe crabs), and wrack material deposited along the strand line (e.g. algae and seagrass). We sampled coquina clams and mole crabs in the swash zone following low tide using cores. We sampled within 27 1-m<sup>2</sup> quadrats that were spread equally across the tidal range on each beach, since these organisms move up and down with the tides (Ellers 1995, Forward et al. 2005). Within each of these we sampled from a total of 9 cores (243 cores per site total). Each core was 20 cm in depth and 10 cm in diameter. Contents from each core were sieved to 1 mm on site and the organisms counted. For the carrion and wrack material, we applied 3 quadrats at each beach on the strandline (50m length X 5m width). We counted all the carrion in those quadrats. For the wrack material, we calculated the approximate surface area covered by wrack (m<sup>2</sup>) within each quadrat. To do this we measured the length and width of each patch of wrack material and then used these measurements to calculate the total surface area in each quadrat covered by wrack. We also measured the burrow density of ghost crabs in three quadrats (20m X 10m) in each site as a proxy for the resource availability for cannibalism. Since these measurements are in different scales, we normalized each type of data collected for each prey item independently. To understand whether the density of prey items of mole crabs, coquina clams and carrion vary with the level of human disturbance, we used separate generalized linear mixed effects models (GLMM) with a Poisson distribution for clams, mole crabs, and carrion, and a general linear mixed effects model for wrack cover. The disturbance level was treated as a fixed factor for all models. To control for the influence of spatio-temporal variations across sites, the latitude of each site and the Julian day on

which it was sampled were treated as random factors. We then applied a Tukey's HSD test for multiple pairwise comparisons of the density of the prey items among the disturbance levels.

#### *7.3.4 Variation in short-term diets*

We examined gut content of all 316 ghost crabs collected as described above and determined the percent contribution of each food type found. We did this by placing the gut content for each crab separately into a Petri dish with grid cells and separating different food types into different grid cells using a dissecting microscope. We then counted the number of grid cells, or portion of a grid cell, covered by each food type. To compare the proportional contribution of different prey items to the diet of crabs from sites with different levels of human disturbance, we used a permutational multivariate analysis of variance (PERMANOVA). Then, we conducted a post-hoc pairwise comparison for the proportional contribution of the diet items among disturbance levels. Similarly, we determined the relationship between the availability in each site and occurrence in the diet of prey items using separate PERMANOVAs for each disturbance levels, followed by a post-hoc pairwise comparison for each prey item. For visual comparisons of the contribution of prey items to diet among and within disturbance levels, we created non-metric multidimensional scaling (nMDS) plots. All analyses and graphs for the short-term diet were conducted using the program PAST. Additionally, we quantified the relative gut fullness visually from 0 (empty) to 4 (full). We used a generalized linear mixed effects model with a Poisson distribution to examine whether gut fullness varied among the levels of human disturbance, treating the levels of human

disturbance as a categorical response variable. We included the latitude of each site and Julian sampling day nested in sampling year as random factors in the model.

#### *7.3.5 Variation in long-term diets*

We examined the long-term diet of ghost crabs using  $\delta^{15}\text{N}$  stable isotope analysis, an indicator of the trophic level (Post 2002, West et al. 2006). We used the muscle tissue of a single walking leg from the crabs collected in 2017 ( $n = 160$ ) from beaches with different levels of human disturbance (Table C.1, Supporting information). We determined  $\delta^{15}\text{N}$  values using an Isoprime mass spectrometer connected via continuous flow to a Euro Vector Elemental Analyzer. To calibrate the system during the analysis, we ran three internal standards for every 40 samples. We applied a linear mixed effects model for the levels of  $\delta^{15}\text{N}$  isotopes, in which we treated the levels of human disturbance as a categorical response variable and we treated the latitude of the study sites as a random factor. Analysis was followed by Tukey' HSD tests for multiple comparisons of the isotope levels between the strengths of human disturbance.

As a second proxy for long term diet, we compared the gut-width:carapace-width ratios of crabs between sites that differed in the strength of human disturbance. The allometric relationship between ghost crab gut-width and carapace width differs with relation to the strength of human disturbance (Griffen et al. 2018). We used the ratio of these two isometric values. A smaller ratio in brachyuran crabs represents a higher quality long-term diet (i.e., generally more animal material in the diet) and the large ratio represents a lower quality of long-term diet (i.e., generally more plant material in the diet) (Griffen & Mosblack 2011). We compared this gut-width:carapace-width ratio between sites with different levels of human disturbance using a linear mixed effects



model, where we once again treated the levels of human disturbance as a categorical response variable and we treated the latitude of the study sites and the Julian sampling day nested in year as random factors.

#### *7.3.6 Tissue analyses*

We quantified the relative physiological condition and the reproductive effort of crabs that were collected in 2016 and in 2017 using two indices: hepatosomatic index (HSI) and gonadosomatic index (GSI), from the dissections described above. These two indices are, respectively, the ratio of the hepatopancreas or the gonad mass and the mass of the rest of the body (Riley et al. 2014). Upon dissection for gut content analysis (described above), we isolated, dried at 70°C, and separately weighed each of these components. We applied two separate linear mixed effects models to understand whether HSI and GSI (females only) values vary among the levels of human disturbance. We included the latitude of the study sites and Julian sampling day nested in the year as random factors, followed by a Tukey's HSD test for multiple pairwise comparisons of the HSI and GSI values among the disturbance levels.

#### *7.3.7 Effects of diet on the physiological condition*

To examine the effects of diet type and amount on the behavior (e.g. aggression level) and physiological condition (e.g. HSI and GSI) of ghost crabs, we collected 60 adult females from Waties Island in August, 2018. These crabs were transported to the Baruch Marine Field Laboratory in Georgetown, South Carolina, USA. We placed the crabs in separate 18.9 L buckets (36.19cm height X 31.11cm diameter) with perforated lids that allows air exchange from the ambient environment to the buckets. We filled each bucket with approximately a 15 cm layer of sand collected from the adjacent Pawley's

Island. We added 50 mL of filtered sea water into the buckets every other day, since ghost crabs require frequent replenishment of the water in their gill chamber to respire (Wolcott, 1976). To prevent an anoxic water layer in the buckets, we made a small hole in the bottom of each.

We initially weighed the crabs to determine the quantity of the food they would be offered. Based on their wet weights, we offered 3% (low) and 10% (high) of their body weight in food every other day. The crabs were kept without food for three days prior to the experiment to standardize their hunger level. We then offered three types of food to our crabs: coquina clams (*D. variabilis*), grouper fish (*Epinephelus* spp.) and beach morning glory (*Ipomoea* spp.). We selected the grouper fish and beach morning glory, because these are local species that are available to the crabs. We collected the clams and the beach morning glory from Pawley's Island on the same day with feeding, and we purchased the fish from local fishermen. Overall, we had 6 different experimental groups (3 different food items and 2 different food amounts), with 10 crabs in each group. We fed crabs every other day with a constant amount and type of food, and uneaten food was removed from the buckets after 24 hours. We opened the clams before being offered to the crabs to make the handling effort of the crabs similar across different food types. The experiment was terminated after 24 days, when all crabs were frozen for further analyses.

To understand whether food type and food portion influence the aggression level of crabs, we measured the behavior using a plastic stick every other day prior to feeding the crabs. We held the plastic stick in front of the crab and observed its response. If the crab attacked to the stick, the behavior was categorized as aggressive. If the crab was

stationary or the crab moved away from the stick, the behavior was categorized as docile (Belgrad & Griffen 2016).

We determined the physiological condition of the crabs using the HSI and GSI. The HSI and GSI values were obtained as explained above (see Tissue analyses). We examined behavior using a generalized linear mixed effects model with a binomial distribution. We treated the crab behavior (aggressive or docile) as a response variable. The food type and proportion of body weight were fixed factors, and the crab ID was a random factor to control for repeated measures. We applied separate 2-way ANOVAs to determine how HSI and GSI were influenced by the food type and food quantity. We conducted a Shapiro-Wilk test of normality and Levene's test for the equality of variances. The data were log-transformed ( $x+1$ ) when needed. Each ANOVA was followed by Tukey' HSD test for multiple pairwise comparisons between experimental groups.

## **7.4. Results**

### *7.4.1 Habitat quality*

All forms of potential food items examined here decreased with increasing levels of human disturbance (Table 7.1, Fig. 7.1). Additionally, we observed horseshoe crabs in the carrion only at pristine sites.

### *7.4.2 Variation in short-term diets*

Based on gut content analysis, crabs consumed mainly the items encountered in our assessment of habitat quality and consumed them according to their relative availability. Proportional contribution of the prey items to the diet of crabs differed among disturbance levels (PERMANOVA,  $F=2.223$ ,  $P=0.035$ , Fig. 7.2, Table C.2,

Supporting information). In general, animal consumption decreased with human impact, while cannibalism and plant consumption increased with human impact (Fig. 7.3a). We also observed consumption on other, rarer, food items, including dead speckled swimming crabs (*Arenaeus cribrarius*) on Pawley's Island and Folly Beach, and a lined sea star (*Luidia clathrate*) on Debordie Island at low tides.

Crabs from pristine sites (PERMANOVA,  $F=23.57$ ,  $P<0.001$ ), from moderately impacted sites (PERMANOVA,  $F=9.432$ ,  $P<0.001$ ), from heavily disturbed sites by people (PERMANOVA,  $F=6.265$ ,  $P<0.001$ ) and from heavily disturbed sites by people and vehicles (PERMANOVA,  $F=3.35$ ,  $P<0.001$ ) appeared to have strong diet item preference (Table C.3, Fig. C.1, Supporting information).

Gut fullness differed based on the human disturbance (Fig. 7.3b). The gut fullness of the crabs from pristine sites was higher than gut fullness of the crabs from moderately impacted sites (GLMM,  $z = -2.317$ ,  $P = 0.0205$ ), from highly impacted sites by people (LMER,  $t = -2.255$ ,  $P = 0.0241$ ) and by people and vehicles (LMER,  $t = -2.784$ ,  $P = 0.0053$ ). However, gut fullness of the crabs did not vary between moderately impacted sites and highly impacted sites by people (Tukey HSD,  $z = -0.014$ ,  $P = 1$ ), and by people and vehicles (Tukey HSD,  $z = -0.823$ ,  $P = 1$ ). Crabs from heavily impacted sites had similar gut fullness (by people vs by people and vehicles, Tukey HSD,  $z = -0.794$ ,  $P = 1$ ).

#### 7.4.3 Variation in long-term diets

Stable nitrogen isotope values of crabs strongly declined with human impact (Fig. 7.4a), suggesting a decrease in mean trophic level with human impact. Specifically, the  $\delta^{15}\text{N}$  values for crabs at pristine sites were significantly higher compared to the crabs from moderately impacted sites (Tukey HSD,  $z = -2.39$ ,  $P = 0.033$ ) highly impacted sites

by people (Tukey HSD,  $z = -3.755$ ,  $P = 0.0006$ ) and by people and vehicles (Tukey HSD,  $z = -6.45$ ,  $P < 0.001$ ). Values for crabs from moderately impacted sites were similar to values for crabs from heavily disturbed sites by people (Tukey HSD,  $z = -1.52$ ,  $P = 0.128$ ), but higher compared to the  $\delta^{15}\text{N}$  isotope values for the crabs from heavily disturbed sites by people and vehicles (Tukey HSD,  $z = -4.395$ ,  $P < 0.001$ ). Vehicles had a significant impact on the  $\delta^{15}\text{N}$  values of the crabs (people vs people and vehicles, Tukey HSD,  $z = -2.78$ ,  $P = 0.016$ ).

Similarly, the gut-width:carapace width ratio increased with human impact, also suggesting a decrease in diet quality (i.e., trophic level) with increasing human impact. Specifically, crabs from pristine sites had smaller ratios compared to the crabs from heavily disturbed sites by people (Tukey HSD,  $z = 3.186$ ,  $P = 0.0057$ ) and by people and vehicles (Tukey HSD,  $z = 5.762$ ,  $P < 0.001$ ). The long-term diet quality of crabs from pristine and moderately impacted sites was similar (Tukey HSD,  $z = 1.654$ ,  $P = 0.162$ ; Fig. 7.4b). The gut-width:carapace width ratios of the crabs from moderately impacted sites were lower compared to the crabs from heavily disturbed sites by people and vehicles (Tukey HSD,  $z = 4.34$ ,  $P < 0.001$ ), but were similar to the crabs from heavily disturbed sites by people (Tukey HSD,  $z = 1.742$ ,  $P = 0.162$ ). The long-term diet quality of crabs was significantly influenced by experiencing the vehicle disturbance (heavily disturbed sites by people vs people and vehicles Tukey HSD,  $z = 2.67$ ,  $P = 0.022$ ).

#### *7.4.4 Variation in physiological conditions*

The standardize hepatopancreas (HSI) of crabs from pristine sites ( $n = 98$ ) was significantly higher compared to the crabs from moderately impacted ( $n = 89$ ; Tukey HSD,  $z = -3.528$ ,  $P = 0.0016$ ), from highly impacted sites by people ( $n = 75$ ; Tukey HSD,

$z = -5.672$ ,  $P < 0.001$ ) and by people and vehicles ( $n = 50$ ; Tukey HSD,  $z = -5.728$ ,  $P < 0.001$ ) (Fig. 7.5a). Crabs from moderately impacted sites were in a better physiological condition compared to the crabs from heavily disturbed sites (by people: Tukey HSD,  $z = -2.295$ ,  $P = 0.0434$ ; by people and vehicles: Tukey HSD,  $z = -2.569$ ,  $P = 0.0305$ ). The presence of vehicles on heavily disturbed sites did not alter the physiological condition of the crabs (Tukey HSD,  $z = -0.404$ ,  $P = 0.686$ ).

We examined a total of 94 adult females to determine the GSI. The GSI values of the crabs was significantly higher at pristine sites ( $n = 32$ ) compared to the crabs from highly impacted sites by people ( $n = 22$ ; Tukey HSD,  $z = -3.001$ ,  $P = 0.013$ ) and by people and vehicles ( $n = 16$ ; Tukey HSD,  $z = -3.504$ ,  $P = 0.0027$ ), but not from moderately impacted sites ( $n = 24$ ; Tukey HSD,  $z = -1.427$ ,  $P = 0.392$ ) (Fig. 7.5b). No significant difference was detected in GSI between crabs from disturbed sites (moderate disturbance vs. heavy disturbance by people Tukey HSD,  $z = -1.511$ ,  $P = 0.392$ ; moderate vs heavy disturbance by people and vehicles: Tukey HSD,  $z = -2.131$ ,  $P = 0.132$ ; heavy disturbance by people vs people and vehicles: Tukey HSD,  $z = -0.736$ ,  $P = 0.461$ ).

#### *7.4.5 Experimental effects of diet on the physiological condition*

Food type and the food portion had significant effects on the crab behavior in our lab experiment. The aggression level of crabs increased as the amount of food offered decreased (GLMM,  $Z = 3.116$ ,  $P = 0.0018$ ). Crabs that were fed with clam were less aggressive than crabs that were fed plants (GLMM,  $Z = -4.999$ ,  $P < 0.001$ ). No difference was found between the aggression levels of the crabs that were fed by clams or fish (GLMM,  $Z = -0.815$ ,  $P = 0.415$ ; Fig. 7.6a).

Food type and food portion had a strong effect on the energy store of the crabs (2-way ANOVA, food type X food portion:  $F = 3.438$ ,  $P = 0.0393$ ). Crabs fed with 10% of their wet body weight of fish had the highest HSI value, and crabs fed with 3% of their wet body weight of plant had the lowest (Fig. 7.6b). Similarly, food type (2-way ANOVA,  $F = 9.985$ ,  $P = 0.0002$ ) and food portion (2-way ANOVA,  $F = 5.336$ ,  $P = 0.0247$ ) had notable influence on the reproduction potential (i.e., GSI) of the crabs. Food type and food portion did not interact with each other in their effects on the GSI (2-way ANOVA,  $F = 1.233$ ,  $P = 0.299$ ; Fig. 7.6c).

## 7.5 Discussion

We have shown notable variations in the short- and long-term diet in the Atlantic ghost crab, *O. quadrata* across habitats with different strengths of human disturbance. We have also shown that this variation in diet may be the mechanistic reason for existing variation in physiological condition. Ghost crabs had a better physiological condition when consuming an animal diet compared to a plant diet, leading to an inverse relationship between physiological condition and human impacts across sites. This decline in physiological condition with human impacts is likely to change the ecology and demographics of ghost crabs, explaining the patterns documented for this bioindicator species on disturbed shores. Results of this study have important implications for this system and for our understanding of human disturbances on natural populations more broadly.

### 7.5.1 Implications for this system

Variations in the short- and long-term diet of ghost crabs overlap with the inverse relationship between the strength of the human disturbance and habitat quality.

Specifically, crabs from disturbed sites have a plant dominated diet and therefore a lower physiological condition compared to crabs from pristine sites that have a primarily carnivorous diet. The frequent cleaning of beaches may be the main cause for this decline in habitat quality. Sites characterized here as heavily disturbed by people and vehicles are mechanically cleaned during nights (Gül & Griffen 2018b) when ghost crabs are most active in foraging. This cleaning reduces the abundance of wrack materials and swash zone organisms (Schlacher et al. 2008, Sheppard et al., 2009). Moreover, the coast of South Carolina experiences relatively low wave energy because of a wide continental shelf (Kana 1988), and this low wave energy leads to limited accumulation of drift material and carrion on the beaches.

Ghost crabs exhibit lower densities at disturbed sites (Schlacher et al. 2016 and citations therein, Gül & Griffen 2018a, 2018b), a pattern with multiple hypothesized explanatory mechanisms, such as higher mortality for individuals with shallower burrows (Schlacher et al. 2007) – though burrow depth tends to increase with the level of human disturbance across sites (Gül & Griffen 2018a), direct crushing by vehicles (Wolcott & Wolcott 1984a), harsh environmental impacts (de Souza et al. 2017), and direct handling by people (Gül & Griffen 2018b). Here we suggest an additional possible mechanism: crab aggression. Previous work shows that crab aggression increases on a diet of algae compared to animal tissue (Belgrad et al. 2017). Similarly, here we have shown that crabs that consumed plant material showed increased aggression compared to crabs that consumed animal material. This suggests that crabs should be more aggressive at disturbed sites where algal/plant diets are more common. Such aggressive behavior could cause higher mortality for crabs at disturbed sites if this increased aggression leads to



greater time outside of burrows, increases other risky behaviors, or increases the rate of cannibalism. This potential scenario could contribute to the smaller observed body size and lower population density at disturbed sites. Further studies are necessary to determine whether the diet-induced changes in aggression that we observed in our experiment are also found in naturally foraging crabs across sites with different diets.

The inverse relationship between the intensity of human disturbance and the physiological condition of crabs across sites suggests a lower growth rate due to a shift in the energetic balance at disturbed sites. The body size and the population abundance of bioindicator species are widely used as readily observable response to the human influences on natural ecosystems (Cortes et al. 2013). However, these observed patterns do not provide any understanding of the mechanistic reason(s) for those demographic changes. Here we have shown that organisms store less energy from their plant-dominated diet at disturbed sites. Moreover, organisms alter their behavior and decrease the amount of energy expended on daily activities (Gül and Griffen in review (a)) and develop smaller claws (Gül and Griffen in review (b)) at sites with heavy human disturbance, suggesting a shortage in energy availability at these sites. Thus, our results here underscore that smaller documented body sizes of organisms on disturbed beaches is likely due to lower habitat quality (i.e., foraging energy intake) that reduces physiological condition.

#### *7.5.2 Broader implications for ecology*

Our study has at least two broader implications for the population ecology of species under human disturbances. First our study demonstrates that cannibalism rates of organisms vary based on human disturbance. Cannibalism rates is lowest under moderate

disturbance and highest under heaviest disturbance. Cannibalism, as a common phenomenon in nature, can stabilize natural populations (Fox 1975, Andersson et al. 2007). Additionally, it may vary based on habitat type (Stevens & Swiney 2005), resource availability (Wise 2006) and physiological stress (Fox 1975), all of which are influenced by human disturbance. Moreover, nitrogen is often a limiting nutrient for organisms that feed on plant-dominated diets (Wolcott & O'Connor 1992), and this limitation can lead to increased cannibalism (Wolcott & Wolcott 1984b). Variation in personality may also directly influence the rate of cannibalism (Start & Gilbert 2017). Our results confirm that cannibalism increases as diet quality in an area decreases and supports the idea that organisms engage in cannibalism to meet specific nutrient needs when these cannot be otherwise met by other food available to them (Wolcott & Wolcott 1984b).

Second, the link between human disturbance and lower reproductive potential suggests that human disturbance may provide a driving mechanistic force leading to source-sink dynamics in coastal areas. Low GSI of crabs at disturbed sites suggests lower population growth rates at disturbed sites compared to pristine sites and that populations at these disturbed sites may not be capable of reproductively supporting their own population growth or stability (Seaward et al. 2018). Thus, populations at heavily disturbed sites may persist due to larval recruitment originating from reproduction at more pristine sites (Pulliam 1988).

In summary, previous studies highlight the advantages of observing demographic changes as a response to the human disturbances (Spellerberg 2005, Cortes et al. 2013, Siddig et al. 2016), yet the mechanistic reason(s) for those changes often remain poorly

understood, and are necessary for forecasting the future conditions of populations as human disturbance continues or intensifies. We have demonstrated that human disturbance leads to reduced physiological and reproductive conditions by altering the diet quality of individuals, which at least partially explains the observed lower population abundance and smaller individual sizes of this bioindicator species.

## 7.6 Tables

Table 7.1. Results of generalized liner mixed-effects model (GLMM) assessing the effects of the fixed factors on the habitat quality (i.e. resource availability) across sites that differ in the strength of the human disturbance. Latitude of the study sites and sampling days (e.g. Julian days) were treated as random factors.

<b>Fixed factor</b>	<b>S.E.</b>	<b>Z- value</b>	<b>P - value</b>
<b>Mole crabs</b>			
<i>A (moderate impact)</i>	0.206	-3.449	0.0005*
<i>B (heavy impact by people)</i>	0.207	-4.611	<0.001*
<i>C (heavy impact by people and vehicles)</i>	0.216	-9.15	<0.001*
<b>Coquina clams</b>			
<i>A</i>	0.142	-4.17	<0.001*
<i>B</i>	0.149	-9.669	<0.001*
<i>C</i>	0.146	-14.142	<0.001*
<b>Carrion</b>			
<i>A</i>	0.37	-2.683	0.007*
<i>B</i>	0.384	-2.854	0.004*
<i>C</i>	0.535	-3.564	0.0003*
<b>Wrack material</b>	<b>S.E.</b>	<b>t-value</b>	<b>P-value</b>
<i>A</i>	0.684	-2.203	0.031*
<i>B</i>	0.968	-2.272	0.026*
<i>C</i>	0.96	-3.236	0.002*

## 7.7 Figures

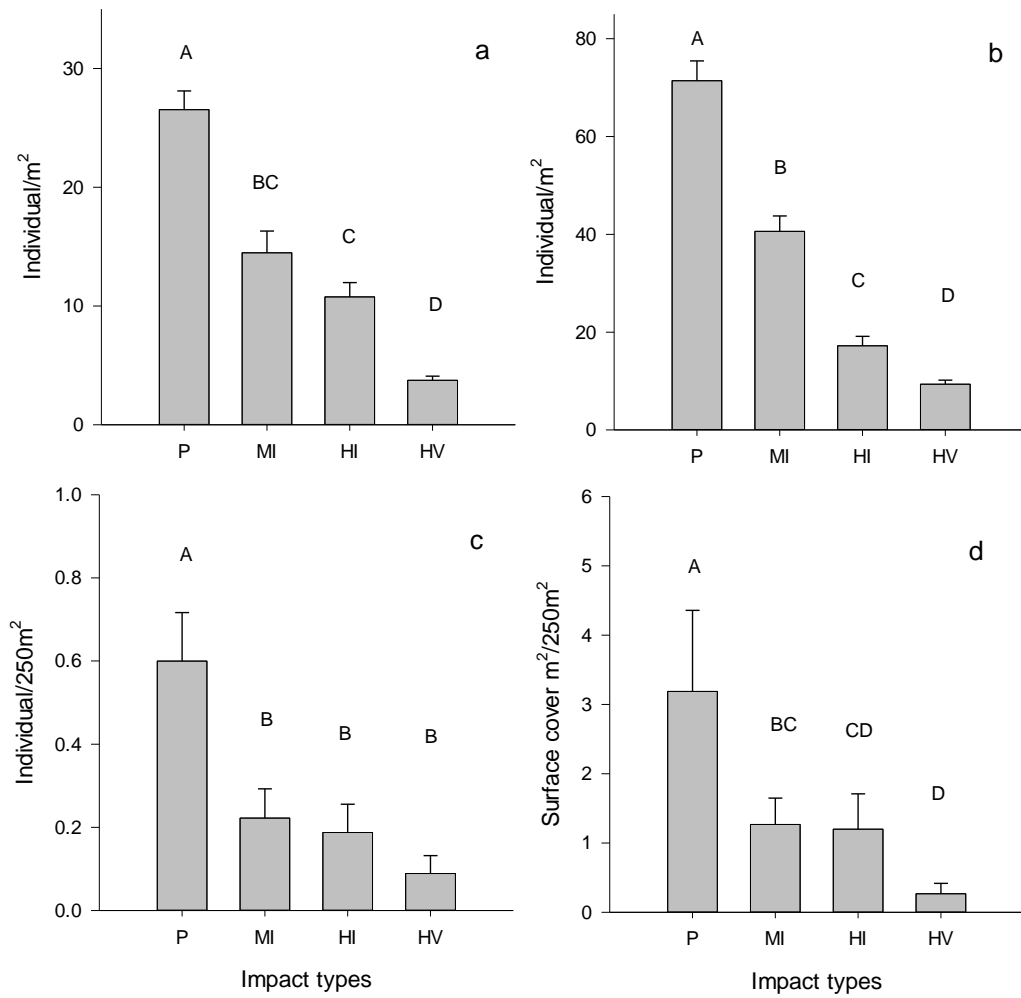


Figure 7.1. Food availability across sites with various levels of human disturbance (mean  $\pm$  S.E.). a) Mole crabs, b) coquina clams, c) carrion, and d) wrack material. Letters above the bars represent the significant differences.

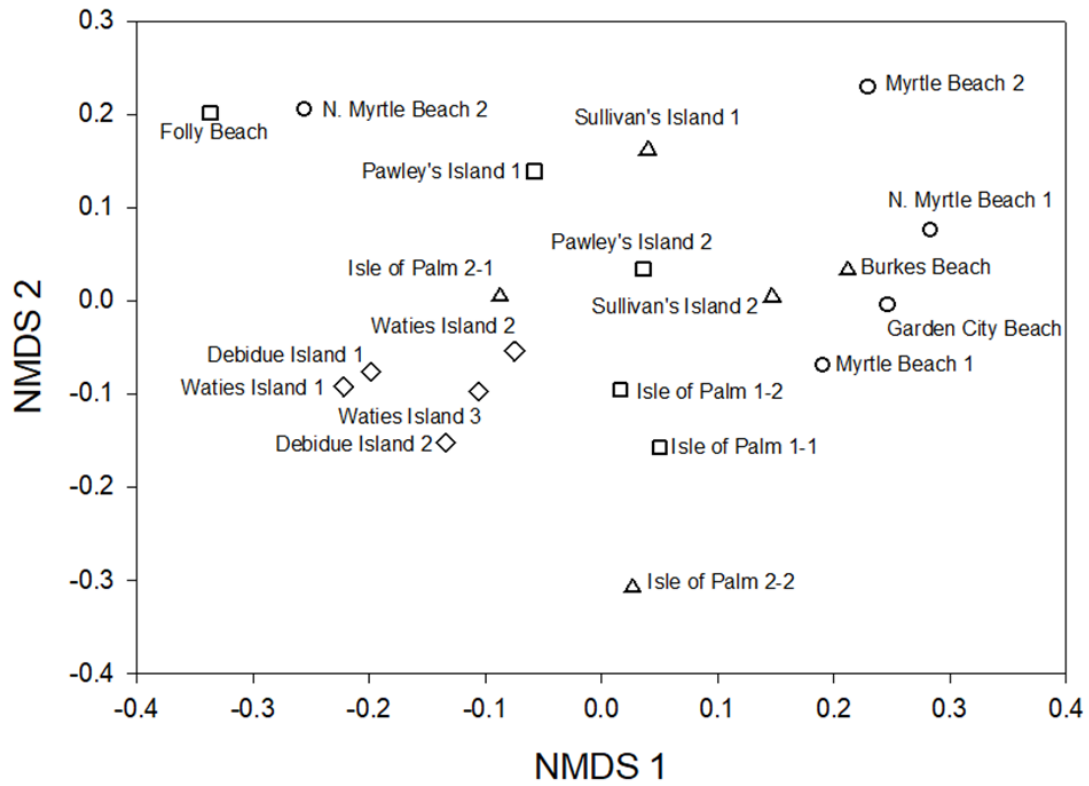


Figure 7.2. NMDS plot for the contribution of prey items to the ghost crab diet among disturbance levels (diamond= pristine sites, square = moderately impacted sites, triangle= highly impacted sites by people, circle = highly impacted site by people and vehicles).

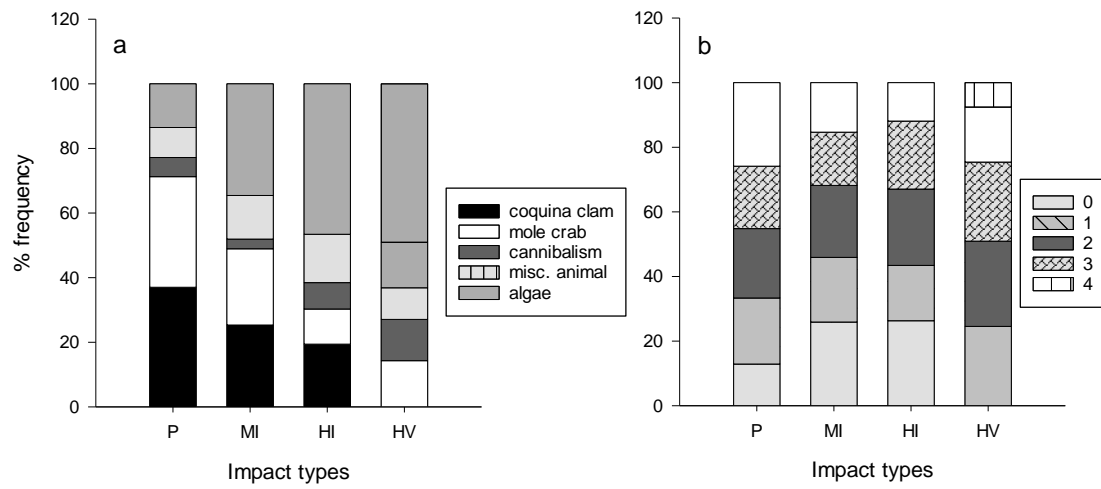


Figure 7.3. Short term diet assessment based on percent frequency of diet items found in the gut contents (a), and gut fullness (b) of ghost crabs across sites with various levels of human disturbance. Letters above the bars indicate the significant difference among disturbance levels.

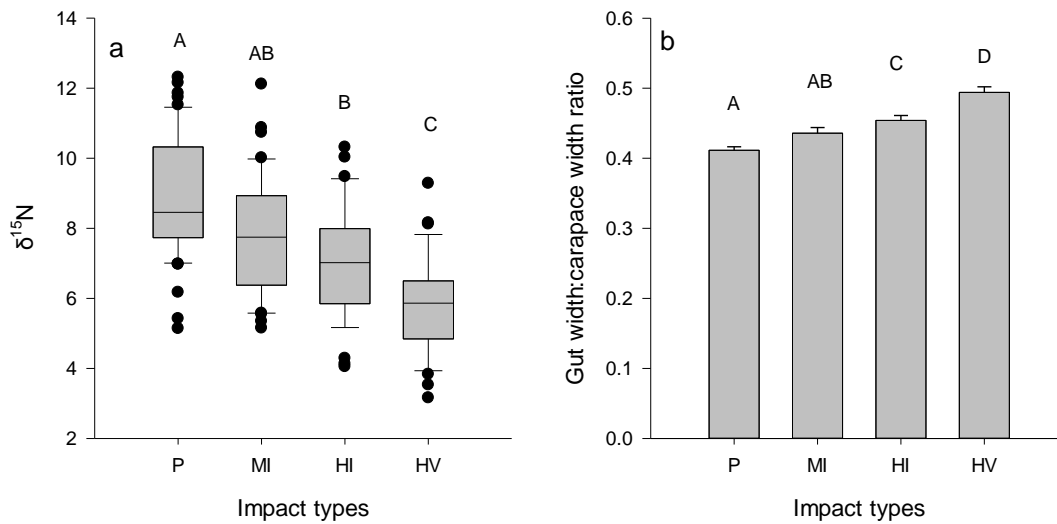


Figure 7.4. Long term diet assessment based on  $\delta^{15}\text{N}$  values from walking leg muscle tissues (a) and the gut-width:carapace-width ratio (b) for ghost crabs across sites with various levels of human disturbance. For each box plot, horizontal lines indicate the median value, boxes encompass the upper and lower quartiles of the data, whiskers encompass 95% of the data, and circles are outliers that fall outside of this range. Letters represent the significant differences.



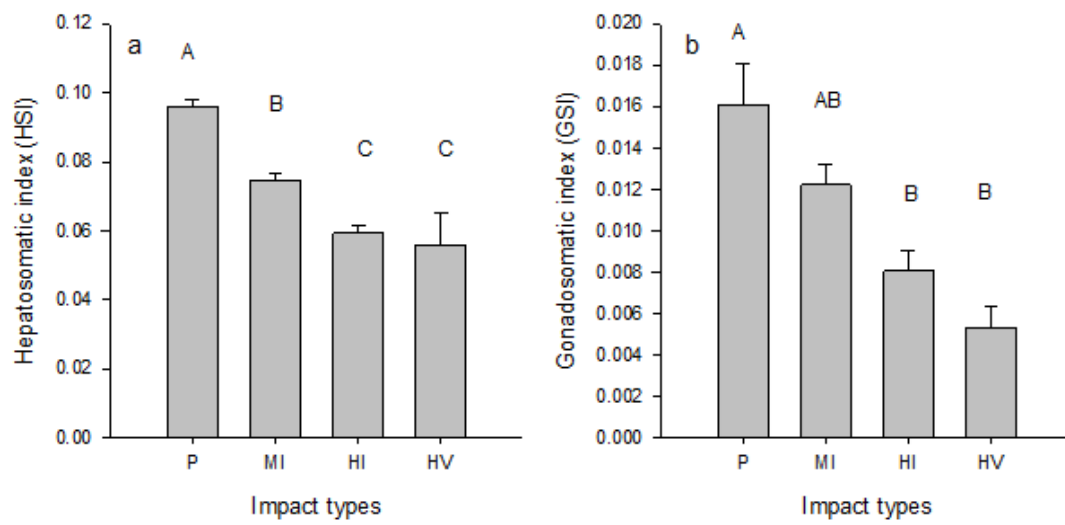


Figure 7.5. Physiological condition as given by the hepatosomatic index (HSI, mean  $\pm$  SE) (a) and the gonadosomatic index (GSI, mean  $\pm$  SE) (b) of ghost crabs from sites that differ in the strength of human disturbance. Letters indicate significant differences.

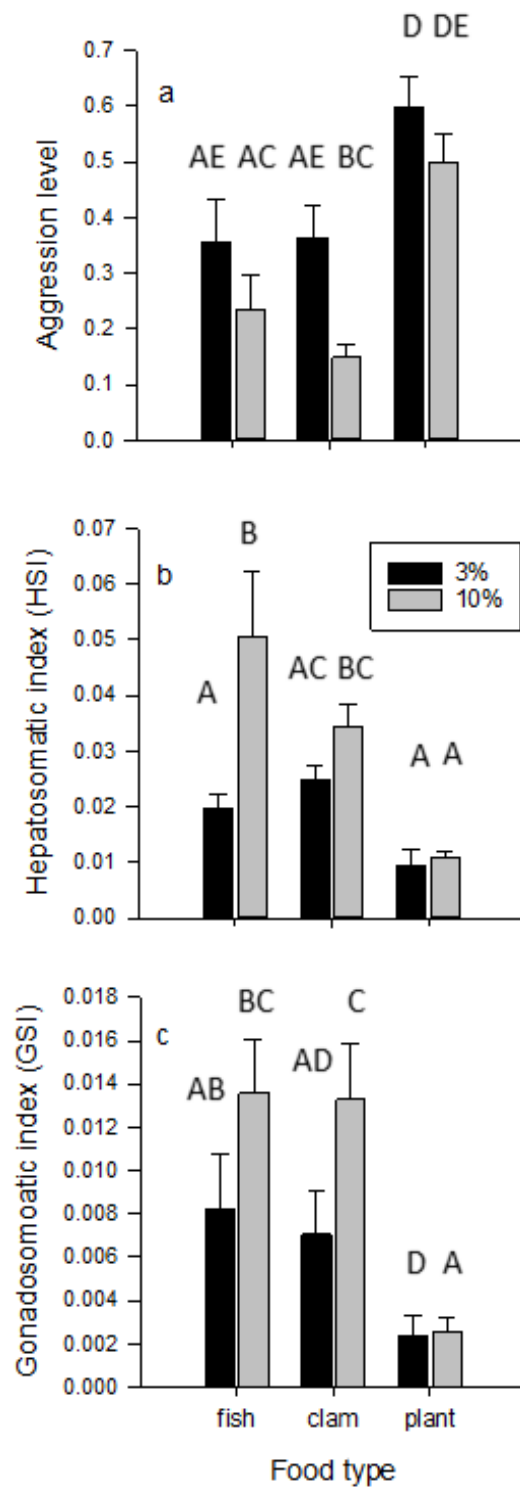


Figure 7.6. Aggression level (a), hepatosomatic index (HSI) (b) and gonadosomatic index (GSI) (c) of crabs were fed with different types and amounts of food in a laboratory experiment. Letters above the bars stand for significant differences.

## 7.8. Literature Cited

- Andersson, J., Bystrom, P., Claessen, D., Perssons, L., & De Roos, A.M. (2007). Stabilization of population fluctuations due to cannibalism promotes resource polymorphism in fish. *American Naturalist*, 169(6), 820- 829. DOI: 10.1086/516846
- Azizi, G., Layachi, M., Akodad, M., Yáñez-Ruiz, D.R., Martín-García, A.I., Baghour, M.,....Moumen, A. (2018). Seasonal variations of heavy metals content in mussels (*Mytilus galloprovincialis*) from Cala Iris offshore (Northern Morocco). *Marine Pollution Bulletin*, 137, 688-694. doi: 10.1016/j.marpolbul.2018.06.052
- Belgrad, B.A., & Griffen, B.D. (2016). The Influence of Diet Composition on fitness of the Blue Crab, *Callinectes sapidus*. *PLoS ONE* 11(1): e0145481. doi:10.1371/journal.pone.0145481
- Belgrad, B.A., Karan, J., & Griffen, B.D. (2017). Individual personality associated with interactions between physiological condition and the environment. *Animal Behaviour*, 123, 277- 284. <https://doi.org/10.1016/j.anbehav.2016.11.008>
- Cardoso, R. S., Barboza, C. A. M., Skinner, V. B., & Cabrini, T. M. B. (2016). Crustaceans as ecological indicators of metropolitan sandy beach health. *Ecological Indicators*, 62, 154–162. <https://doi.org/10.1016/j.ecolind.2015.11.039>
- Cortes, R.M.V., Hughes, S.J., Pereira, V.R., & Varandas, S.D.P. (2013). Tools for bioindicator assessment in rivers: the importance of spatial scale, land use patterns and biotic integration. *Ecological Indicators*, 34, 460- 477. <https://doi.org/10.1016/j.ecolind.2013.06.004>

- Davenport, J., & Davenport, J. L. (2006). The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine, Coastal and Shelf Science*, 67(1-2), 280- 292. <https://doi.org/10.1016/j.ecss.2005.11.026>
- Defeo, O., & de Alava, A. (1995). Effects of human activities on long-term trends in sandy beach populations: the wedge clam *Donax hanleyanus* in Uruguay. *Marine Ecology Progress Series*, 123, 72–82.
- de Souza, G.N., Oliveira, C.A.G., & Tardem, A.S. (2017). Counting and measuring ghost crab burrows as a way to assess the environmental quality of beaches. *Ocean and Coastal Management*, 140, 1–10.
- Ellers, O. (1995). Behavioral control of swash-riding in the *Donax variabilis*. *Biological Bulletin*, 189, 120- 127.
- Feary, D.A., McCormick, M.I., & Jones, G.P. (2009). Growth of reef fishes in response to live coral cover. *Journal of Experimental Marine Biology and Ecology*, 373(1), 45- 49. doi:10.1016/j.jembe.2009.03.002
- Fisher, J. & Tevesz, M. (1979). Within-habitat spatial patterns of *Ocypode qudrata* (Fabricius) (Decapoda Brachyura). *Crustaceana*, 5, 31- 36.
- Forward, R.B., Diaz, H., Cohen, J.H. (2005). The tidal rhythm in activity of the mole crab *Emerita talpoida*, *Journal of the Marine Biological Association of the United Kingdom*, 85 (4), 895- 901.
- Franklin, A.B., Anderson, D.R., Gutiérrez, R.J., Burnham, K.P. (2000). Climate, habitat quality, and fitness in northern spotted owl populations in Northwestern California. *Ecological Monographs*, 70(4), 539- 590.

- Fox, L.R. (1975). Cannibalism in natural populations. *Annual Review of Ecological Systems* 6: 87-106.
- González, S. A., Yáñez-Navea, K., & Muñoz, M. (2014). Effect of coastal urbanization on sandy beach coleoptera *Phaleria maculate* (Kulzer, 1959) in northern Chile. *Marine Pollution Bulletin*, 83(1): 265–274. <https://doi.org/10.1016/j.marpolbul.2014.03.042>
- Griffen, B.D. (2014). Linking individual diet variation and fecundity in an omnivorous marine consumer. *Oecologia*, 174, 121- 130. DOI 10.1007/s00442-013-2751-3
- Griffen, B.D., & Norelli, A.P. (2015). Spatially variable habitat quality contributes to within-population variation in reproductive success. *Ecology and Evolution*, 5(7), 1474-1483. doi: 10.1002/ece3.1427
- Griffen, B.D., & Mosblack, H. (2011). Predicting diet and consumption rate differences between and within species using gut ecomorphology. *Journal of Animal Ecology*, 80, 854- 863. doi: 10.1111/j.1365-2656.2011.01832.x
- Griffen, B.D., Cannizzo, Z.J., & Gül, M.R. (2018). Ecological and evolutionary implications of allometric growth in stomach size of brachyuran crabs. *PLoS ONE* 13(11): e0207416. <https://doi.org/10.1371/journal.pone.0207416>
- Griffen, B.D., & Riley, M.E. (2015). Potential impacts of invasive crabs on one life history strategy of native rock crabs in the Gulf of Maine. *Biological Invasions*, 17, 2533-2544. DOI 10.1007/s10530-015-0890-7
- Griffiths, J. R., Kadin, M., Nascimento, F. J. A., Tamelander, T., Törnroos, A., Bonaglia, S.,.....Winder, M. (2017). The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. *Global Change Biology*, 23, 2179- 2196. doi: 10.1111/gcb.13642

Gül, M. R., & Griffen, B. D. (2018a). Impacts of human disturbance on ghost crab burrow morphology and distribution on sandy shores. PLoS ONE 13(12): e0209977. <https://doi.org/10.1371/journal.pone.0209977>

Gül, M. R., & Griffen, B. D. (2018b). A reliable bioindicator of anthropogenic impact on the coast of South Carolina. Southeastern Naturalist. 2018; 17(2): 357–364. <https://doi.org/10.1656/058.017.0217>

Gül, M. R., & Griffen, B. D. (in review(a)). Burrowing behavior and burrowing energetics of a bioindicator under human disturbance. Ecology and Evolution.

Gül, M. R., & Griffen, B. D. (in review(b)). Changes in claw morphology of a bioindicator species across habitats that differ in human disturbance. Hydrobiologia.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., .....Watson, R. (2008). A global map of human impact on marine ecosystems. Science, 319, 948- 952. VAR

Jokimäki, J., Suhonen, J., Jokimäki-Kaisanlahti, M. L., & Carbó-Ramírez, P. (2016). Effects of urbanization on breeding birds in European towns: Impacts of species traits. Urban Ecosystems, 19(4), 1565- 1577. doi: 10.1007/s11252-014-0423-7

Kana, T.W. (1988). Beach erosion in South Carolina. South Carolina Sea Grant Consortium.

Kiskaddon, E., ChernickyK., & Bell, S. (2019). Resource use by and trophic variability of *Armases cinereum* (Crustacea, Brachyura) across human-impacted mangrove transition zones. PLoS ONE, 14(2): e0212448. <https://doi.org/10.1371/journal.pone.0212448>

- Kuklina, I., Kouba, A., & Kozák, P. (2013). Real-time monitoring of water quality using fish and crayfish as bio-indicators: a review. *Environmental Monitoring and Assessment*, 185(6), 5043- 5053. doi: 10.1007/s10661-012-2924-2
- Lim, S.S.L., Yong, A.Y.P., & Christy, J.H. (2016). Ontogenetic changes in diet and related morphological adaptations in *Ocypode gaudichaudii*. *Invertebrate Biology*, 135(2), 117-126.
- Loreau, M., Daufresne, T., Gonzalez, A., Gravel, D., Guichard, F., Leroux, S.J.,.....Mouquet, N. (2013). Unifying sources and sinks in ecology and Earth sciences. *Biological Reviews*, 88, 365-379. doi: 10.1111/brv.12003
- Lucrezi, S. & Schlacher, T. A. (2014). The ecology o ghost crabs-a review. *Oceanography and Marine Biology- Annual Review*, 52, 201- 256.
- Lyon, J.P., Bird, T.J., Kearns, J., Nicol, S., Tonkin, Z., Todd, C.R.,.....Bradshaw, C.J.A. (2019). Increased population size of fish in a lowland river following restoration of structural habitat. *Ecological Applications*, 29(4), e01882.  
<https://doi.org/10.1002/eap.1882>
- Maxwell, S.M., Hazen, E.L., Bograd, S.J., Halper, B.S., Breed, G.A., Nickel, B.....Costa, D.P. (2013). Cumulative human impacts on marine predators. *Nature Communications*, 4, 1-9. doi:10.1038/ncommsS3688
- Morrow, K., Bell, S.S., & Tewfik, A. (2014). Variation in ghost crab trophic links on sandy beaches. *Marine Ecology Progress Series*, 502, 197- 206.
- Post, D.M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83(3), 703- 718.

- Pulliam, H.R. (1988). Sources, sinks, and population regulation. *American Naturalist*, 132, 652–661. VAR
- Rasmussen, J.E., & Belk, M.C. (2012). Dispersal behavior correlates with personality of a North American fish. *Current Zoology*, 58(2), 260- 270.
- Requera, P., Couceiro, L., & Fernández, N. (2018). A review of the empirical literature on the use of limpets *Patella* spp. (Mollusca: Gastropoda) as bioindicators of environmental quality. *Ecotoxicology and Environmental Safety*, 148, 593- 600.  
<https://doi.org/10.1016/j.ecoenv.2017.11.004>
- Riley, M.E., Vogel, M., & Griffen, B.D. (2014). Fitness-associated consequences of an omnivorous diet for the mangrove tree crab *Aratus pisonii*. *Aquatic Biology*, 20, 35- 43.  
doi: 10.3354/ab00543
- Seward, A., Ratcliffe, N., Newton, S., Caldow, R., Piec, D., Morrison, P.,.....Bolton, M. (2018). Metapopulation dynamics of roseate terns: Sources, sinks and implications for conservation management decisions. *Journal of Animal Ecology*, 88, 138-153. DOI: 10.1111/1365-2656.12904
- Schlacher, T. A., Lucrezi, S., Connolly, R. M., Peterson, C. H., Gilby, B. L., Maslo, B., ..... Schoeman, D. S. (2016). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. *Estuarine, Coastal and Shelf Science*, 169, 56- 73. <https://doi.org/10.1016/j.ecss.2015.11.025>
- Schlacher, T.A., Thompson, L.M.C., & Walker, S.J. (2008). Mortalities caused by off-road vehicles (ORVs) to a key member of sandy beach assemblages, the surf clam *Donax deltoides*. *Hydrobiology* 610, 345–350.



Schlacher, T.A., Thompson, L., & Price, S. (2007). Vehicles versus conservation of invertebrates on sandy beaches: mortalities inflicted by off-road vehicles on ghost crabs.

Marine Ecology, 28(3), 354–367. <https://doi.org/10.1111/j.1439-0485.2007.00156.x>

Schooler, N.K., Dugan, J.E., & Hubbard, D.M. (2019). No lines in the sand: Impacts of intense mechanized maintenance regimes on sandy beach ecosystems span the intertidal zone on urban coasts. Ecological Indicators, 106, 105457.

<https://doi.org/10.1016/j.ecolind.2019.105457>

Siddig, A.A.H., Ellison, A.M., Ochs, A., Villar-Leeman, C., & Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in ecological indicators. Ecological Indicators, 60, 223-230.

[doi:10.1016/j.ecolind.2015.06.036](https://doi.org/10.1016/j.ecolind.2015.06.036)

Sheppard, N., Pitt, K. A., & Schlacher, T. A. (2009). Sub-lethal effects of off-road vehicles (ORVs) on surf clams on sandy beaches. Journal of Experimental Marine Biology and Ecology, 380, 113–118. <https://doi.org/10.1016/j.jembe.2009.09.009>

Start, D., & Gilbert, B. (2017). Predator personality structures prey communities and trophic cascades. Ecology Letters, 20, 366- 374. doi: 10.1111/ele.12735

Stevens, B.G., & Swiney, K.M. (2005). Post-settlement effects of habitat type and predator size on cannibalism of glaucothoe and juveniles of red king crab *Paralithodes camtschaticus*. Journal of Experimental Marine Biology and Ecology, 321, 1-11.

[doi:10.1016/j.jembe.2004.12.026](https://doi.org/10.1016/j.jembe.2004.12.026)

Tewfik, A., Bell, S.S., McCann, K.S., & Morrow, K. (2016). Predator diet and trophic position modified with altered habitat morphology. PLoS ONE, 11(1), e0147759.

[doi:10.1371/journal.pone.0147759](https://doi.org/10.1371/journal.pone.0147759)

- Vasconcelos, R.P., Eggleston, D.B., Pape, O.L., & Tulp, I. (2014). Patterns and processes of habitat-specific demographic variability in exploited marine species. *ICES Journal of Marine Science*, 71(3), 638- 647. doi:10.1093/icesjms/fst136
- Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of the Earth's ecosystems. *Science*, 277, 494–499.
- West, J.B., Bowen G.J., Cerling, T.E., & Ehleringer, J.R. (2006). Stable isotopes as one of nature's ecological recorders. *Trends in Ecology & Evolution*, 21(7), 408- 414.  
<https://doi.org/10.1016/j.tree.2006.04.002>
- Wise, D.H. (2006). Cannibalism, food limitation, intraspecific competition, and the regulation of spider populations. *Annual Review of Entomology*, 51:441–465.
- Wolcott, T.G. (1976). Uptake of soil capillary water by ghost crabs. *Nature*, 264, 756-757.
- Wolcott, T.G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fab.) on an ocean beach: scavengers or predators? *Journal of Experimental Marine Biology and Ecology*, 31, 67–82.
- Wolcott, T.G., & Wolcott, D.L. (1984a). Impact of off-road vehicles on macroinvertebrates on a Mid-Atlantic beach. *Biological Conservation*, 29(3), 217- 240.  
[https://doi.org/10.1016/0006-3207\(84\)90100-9](https://doi.org/10.1016/0006-3207(84)90100-9)
- Wolcott, D.L., & Wolcott, T.G. (1984b). Food quality and cannibalism in the red land crab, *Gecarcinus lateralis*. *Physiological Zoology*, 57(3), 318- 324.
- Wolcott, D.L., & O'Connor, N.J. (1992). Herbivory in crabs: adaptations and ecological considerations. *American Zoologist*, 32, 370- 381.

## CHAPTER 8

### GENERAL CONCLUSION

#### 8.1. Conclusion

In this dissertation we investigated the effects of human disturbance on the population demographics, physiology, and ecology of a bioindicator species by addressing six key topics:

CHAPTER 2 identified the variations in the population demographics of *O. quadrata* between sites that differ in the strength of the human disturbance by applying an indirect burrow counting technique, which is a reliable indicator of population density (Schlacher et al. 2016). Additionally, the opening diameter of the ghost crab burrows is a proxy of the crab size (Wolcott 1978). The density of ghost crab burrows declined with increased the strength of the human disturbance. Moreover, the size of ghost crab burrow openings was inversely related to the strength of the human disturbance. Additionally, human disturbance changed the sex ratio of the populations by playing a selective role on the male individuals. This chapter showed that examining the burrow density *O. quadrata* using the indirect counting technique provides useful and reliable information for the degree of ecological changes on the sandy shores due to human disturbance. Additionally, human disturbance may have important implications on the reproductive characteristics of populations by altering their sex ratio.

CHAPTER 3 furthered finding in CHAPTER 2 by showing the variations in burrowing characteristics and distribution within beaches of *O. quadrata* between sites that differ in the strength of the human disturbance. This chapter showed that species alter their site distribution under human influence. Specifically, although ghost crab populations show a more homogenous distribution at the sites with moderate disturbance and no disturbance, they often prefer burrowing at the edges of the beaches at heavily disturbed sites to potentially experience less disturbance. In order to explain the variations in the distribution patterns of crabs between less impacted and highly impacted sites, this chapter further showed that the level of the relative humidity varied between sites with relation to the human disturbance. The level of humidity is important for semi-terrestrial crabs, since they need to moisten their gills for respiration, frequently (Wolcott 1976). Additionally, this chapter demonstrated that crabs at disturbed sites created shorter, deeper and steeper burrows at disturbed sites and the relative humidity inside the burrows show similar values but the temperatures in the burrow vary in the same sites, suggesting that burrow depth is determined by the relative humidity and ghost crabs can tolerate the variations in the temperature when they have can reach enough moisture. By conducting field surveys, this chapter provides evidence that human disturbance lead to changes in the behavior and distribution pattern of organisms, which might be the consequences of the shifts in the energetic balance of organisms under human disturbance.

CHAPTER 4 investigated whether human disturbances affect the ability of *O. quadrata* to persist when they experience extreme natural disturbances. By conducting through pre- and post-hurricane field surveys, this chapter showed that human

disturbances make the *O. quadrata* populations less resilient against natural disturbances. After the hurricane, all populations were influenced the storm effects, substantially. This chapter further showed that larger individuals were disproportionally influenced by Hurricane Matthew. Moreover, the distribution patterns pre- and post-hurricane were substantially different. This chapter furthered the findings of CHAPTER 3 on the distribution pattern. While, the larger individuals frequently occupy the upper parts of beaches, smaller crabs burrow next to the strand line on the beaches with human disturbance before the hurricane. However, after the hurricane, the smaller individuals occupied the upper parts of the beaches and the burrows distributed more homogenously. Some studies suggested that intraspecific interactions may play a role in the distribution pattern of the ghost crabs (Lucrezi & Schlacher 2014). Therefore, this chapter has answered a long-lasting question regarding to the distribution of *O. quadrata* providing evidence that intraspecific interactions are main factors that determine the distribution pattern that is observed at disturbed sites. This chapter suggests that human disturbance may shift the strength of the species interactions, which determine the distribution pattern of the organisms.

CHAPTER 5 demonstrated that human disturbances play a selective role on the morphology of individuals that differ in sex. Claw size of *O. quadrata* individuals showed variations between sites that differ in the strength of the human disturbance. Claw size of individuals from disturbed sites were notably lower compared to the individuals from pristine sites. Moreover, the strength of the human disturbance played an important role on claw size variations between male and female crabs. Additionally, the claw closing force, a measure of the claw height (Lee 1993, Yamada & Boulding

1998, Sneddon et al. 2000), fluctuated in male crabs with relation to the strength of the human disturbance. Female individuals, on the other hand, had similar claw closing force across the levels of human disturbances. A higher quality of diet might be the reason in the variation of the claw size (Smith & Palmer 1994), which may be the reason for the observations of this chapter. Additionally, individuals living at sites with higher population densities may become better competitors (Calsbeek 2009), which may lead to a shift in the morphological characteristics of the organisms, suggesting that crabs at pristine sites are better competitors due to the higher population abundance and they obtain a better diet compared to the crabs from disturbed sites. The variation in the claw sizes between male and female crabs are likely due to the differences in the growth rate of the individuals from different sexes (Haley 1969, Hartnoll 1974). Ultimately, this chapter suggests that may have important implications on the ability of being a better competitor and on the diet items that organisms consume by altering the claw size.

CHAPTER 6 explored how the human disturbance can alter the burrowing behavior and burrowing energetics of *O. quadrata*. This chapter demonstrated that *O. quadrata* can use the same burrow for a longer time at the sites with human disturbance compared to the crabs from pristine sites. This chapter further showed that the burrows of *O. quadara* can persist for a longer time at disturbed sites compared to the burrows at pristine sites and the rate of the sand compaction is an important determinant of this time. Additionally, across all disturbance levels, the larger individuals had a higher burrow fidelity compared to the smaller crabs. This chapter also demonstrated that ghost crabs spends a substantial amount of energy for burrowing. The glycogen level in the digging leg muscle tissues dropped by almost 50% after 2 hours of digging. By combining the

data of burrow size collected in CHAPTER 3, the energetic equivalent of glycogen level in the digging leg muscle tissue, burrow longevity and the portion of the year when crabs are active (Christoffers 1986), this chapter showed that ghost crabs spend a much higher amount of energy for their burrowing behavior at pristine sites compared to the disturbed sites. Ultimately, this chapter suggests that individuals living under human disturbance change their daily activities and behaviors to save energy.

CHAPTER 7 established a mechanistic understanding of the variation in the habitat quality between sites that differ in the strength of the human disturbance and the effects of the habitat quality on the *O. quadrata* populations by examining the short and long-term diets and physiological condition and the reproductive potential of the crabs collected from sites. Additionally, this chapter examined the behavioral, physiological and reproductive consequences of the various diet items and various proportions of the diet. This chapter showed that human disturbance has a substantial influence on the habitat quality on the basis of diet type and availability. Ghost crabs prefer a more carnivory diet on sandy shore by disproportionally feeding on mole crabs and coquina clams probably due to their higher caloric content (Wolcott 1978). Pristine sites had the most available preferred diet items for *O. quadrata*, in contrast, heavily disturbed sites had the least. A similar pattern was observed during the short-term diet analysis, which crabs had a higher quality diet with approximately 90% animal tissues, in contrast, crabs from heavily disturbed sites had a plant tissue dominated diet. Similarly, long-term diet analysis revealed that crabs from pristine sites occupy a higher position in the trophic level compared to the crabs from disturbed sites. This chapter further showed that crabs from pristine sites had a better physiological condition and they invested more energy for

their reproduction compared to the crabs from disturbed sites, suggesting a shift in bottom-up effects. Additionally, higher reproductive potential of the crab from pristine sites and lower reproductive potential of the crabs from heavily disturbed sites suggested that pristine sites are the sources for the persistence of the populations at heavily disturbed sites. Results of the laboratory work provided further evidence for this hypothesis. Laboratory work revealed that crabs that were fed with plant diet showed a more aggressive behavior, lower physiological condition and lower reproductive potential compared to the crabs that were fed by animal diet. Ultimately, this chapter suggested that the lower habitat quality at disturbed sites can be the mechanistic reason for the lower individual body sizes of the bioindicator species.

Collectively, this dissertation provides important insights that expand our understanding of the mechanistic reasons for the responses of bioindicator species to the human disturbances. It explored the influences of the human disturbance on a variety of important aspects of ecology, physiology, morphology and energetics of a bioindicator species to show the potential mechanistic reasons of the variations in the population demographics under human disturbance. This dissertation documented the shifts in the sex-ratio and population demographics (CHAPTERS 2, 3, 4), and the behavior and distribution within site under human (CHAPTER 3) and natural disturbances (CHAPTER 4). It further documented how the possible shortage in the food supply at disturbed sites alters the morphological characteristics (CHAPTER 5). Additionally, this dissertation documented the energetic consequences of the behavioral changes of the organisms under human disturbance (CHAPTER 6). Finally, it showed that human



disturbance influences population demographics, physiological conditions and reproductive potentials of the bioindicator species by altering the habitat quality (CHAPTER 7). This dissertation explored a variety of ecological and physiological themes of a bioindicator species and its results highlighted that energy shortages due to the diet availability and the quality might be the mechanistic reason behind the responses of bioindicator species to the human disturbances.

## **8.2. Literature Cited**

Calsbeek, R. (2009). Experimental evidence that competition and habitat use shape the individual fitness surface. *Journal of Evolutionary Biology* 22:97–108.

doi:10.1111/j.1420- 9101.2008.01625.x

Christoffers, E. W. (1986). Ecology of the ghost crab *Ocypode quadrata* (Fabricius) on Assateague Island, Maryland and the impacts of various human uses of the beach on their distribution and abundance. PhD thesis, Michigan State University, East Lansing, MI, USA.

Haley, S.R. (1969). Relative growth and sexual maturity of the Texas ghost crab, *Ocypode quadrata* (Fabr.). (Brachyura, Ocypodidae). *Crustaceana*. 17:285–297

Hartnoll, R.G. (1974). Variation in growth pattern between some secondary sexual characters in crabs (Decapoda Brachyura). *Crustaceana*, 27:131–136

Lee, S.Y., (1993). Chela height is an acceptable indicator of chela strength in *Carcinus maenas* (Linnaeus, 1758) (Decapoda, Brachyura). *Crustaceana*, 65:115-116

Lucrezi, S. & Schlacher, T. A. (2014). The ecology o ghost crabs-a review.

Oceanography and Marine Biology- Annual Review, 52, 201- 256.

Schlacher, T. A., Lucrezi, S., Connolly, R. M., Peterson, C. H., Gilby, B. L., Maslo, B., ..... Schoeman, D. S. (2016a). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. Estuarine, Coastal and Shelf Science, 169, 56- 73. <https://doi.org/10.1016/j.ecss.2015.11.025>

Sneddon, L.U., F.A. Huntingford, A.C. Taylor & J.F. Orr, (2000). Weapon strength and competitive success in the fights of shore crabs (*Carcinus maenas*). Journal of Zoology, 250:397- 403

Wolcott TG. (1976) Uptake of soil capillary water by ghost crabs. Nature, 264: 756–757. <https://doi.org/10.1038/264756a0>

Wolcott, T.G. (1978). Ecological role of ghost crabs, *Ocypode quadrata* (Fab.) on an ocean beach: scavengers or predators? Journal of Experimental Marine Biology and Ecology, 31, 67–82.

Yamada, S.B. & E.G. Boulding, (1998). Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. Journal of Experimental Marine Biology and Ecology, 220:191-211

## APPENDIX A:

### METHODOLOGICAL SUPPLEMENT FOR CHAPTER 6

#### **A.1 Assessing Geomorphological Characteristics of Sandy Beaches**

Geomorphological characteristics of sandy beaches (e.g. sand compaction, grain size) of sandy beaches are important determinants of burrowing preference and behavior (Lim et al. 2011, Dixon et al. 2015). Thus, we measured the sand compaction rate and the sand grain size. Sand compaction rate was measured using a pocket penetrometer with the adapter foot (AMS, E-280) on the first day of the longevity study. We measured the sand compaction rate three times in each quadrat and used the mean value. For the sand grain analysis, we collected three 500 g sand samples from each study sites. These were dried at 70 °C for 24 and were then passed through a series of sieves of a variety of sizes (Folk 1980).

Burrow longevity and burrow density are inversely related to each other in ghost crab populations (Hughes 1966). Thus, we measured the burrow density by observing the number of burrows in the quadrats on the first day of the longevity experiment.

#### **A.2 Analysis of Burrow Volume**

To obtain the volume of the burrows, we poured a 2:1 mixture of plaster of Paris and freshwater into the burrows (Chan et al. 2006). After about 30 minutes, we excavated the casts using a shovel and tagged them for later measurement. In the laboratory, a small subsample of the same plaster mixture was prepared in a container whose volume was known. All casts and this subsample were then dried to constant weight at 70 °C. The density of the mixture was determined by using the weight of the subsample of known volume. We then weighed the burrow casts and obtained the volume of each by dividing this density by the mass of each burrow.

### A.3 Literature Cited

- Chan, B.K.K., Chan, K.K.Y., & Leung, P.C.M. (2006). Burrow architecture of ghost crab *Ocypode ceratophthalma* on a sandy shore in Hong Kong. *Hydrobiologia*, 560(1): 43–49.  
<https://doi.org/10.1007/s10750-005-1088-2>
- Dixon, R.W., Peters, S.L., & Townsend, C.G. (2015). Burrowing preferences of Atlantic ghost crab, *Ocypode quadrata*, in relation to sand compaction in Padre Island National Seashore, Texas. *Physical Geography*, 36(3), 188–201.
- Folk, R.L. (1980). Petrology of sedimentary rocks. Hemphill Publishing Company.
- Hughes, D. A. (1966). Behavioural and ecological investigations of the crab *Ocypode ceratophthalmus* (Crustacea: Ocypodidae). *Journal of Zoology*, 150, 129–143.
- Lim, S.S., Yong, A.Y., & Tantichodok, P. (2011). Comparison of burrow morphology of juvenile and young adult *Ocypode ceratophthalmus* from Sai Kaew, Thailand. *Journal of Crustacean Biology* 31, 59–65.

APPENDIX B:  
SUPPLEMENTAL TABLES FOR CHAPTER 6

Table B.1. Levels and score used to estimate UI for South Carolina sandy beaches (modified from González et al., 2014).

Scores	Proximity to urban centers	Buildings on the sand and dunes	Beach cleaning the sand	Number of vehicles on	Visitor frequency	Infrastructure
0	Totally rural. Kilometers away from city center. No access by car.	No building close to beach	Never cleaned mechanically	No access to the beach by vehicle.	No visitor at all.	No infrastructure.
1	Totally rural. A few kilometers away from city center. No access by car. Pathway exists	Building close to beach about a few kilometers.	Beach mechanically cleaned if needed (may be once a while)	No vehicle and tire traces observed.	Almost no visitor. Pathway exists.	Only pathway and very limited access.
2	Located close to urban center. Some impact of urban center like noise or light	Buildings exist but far away	Beach mechanically cleaned a few times in a year. May be once a month.	Access by vehicle is very limited. Some irregular traces observed.	Scarce visitor.	Only parking lot.
3	Close to urban center. Accessed by car. Vehicles pass nearby.	Buildings close to the beach but not on the dunes or sand.	Beach mechanically cleaned more frequent than once a month.	Moderately vehicle access. Some irregular traces.	Mostly local visitors. Has a public access.	Parking lot and some amenities. Good access.
4	Next to the urban center. Direct access by car. Direct impact by noise and light.	Building just next to the beach, but not on the dunes or sand.	Beach continuously mechanically cleaned. May be a few times a week.	Easily vehicle access. Dense traces. Vehicles observed.	Public access. High visitor demand.	Good access. Good infrastructure.
5	The city is next to the beach. Direct access by car and direct heavy impact by light and noise.	There are building on the dunes and/or sand.	Beach mechanically cleaned every day.	Advance access to the beach by vehicle. Different types of vehicles observed.	Public access. Very high visitor demand. Destination of long vacations.	Very good access and infrastructure including security and life guards.

Table B.2. Geo-morphological variables and burrow density of ghost crabs on study sites. In the table, P represents the pristine sites, MI represents the moderately impacted sites, HI represents the highly impacted sites by people, and HV represents the highly impacted sites by people and vehicles.

Site	Latitude Longitude	Impact types	Sand compaction (kg cm <sup>-2</sup> )	Sand grain size (mm)	Burrow density (indv. m <sup>-2</sup> )
Waties Island 1	33°50'52" N 78°33'48" W	P	0.051	0.196	0.61
Waties Island 2	33°50'45" N 78°34'33" W	P	0.043	0.211	0.77
Waties Island 3	33°50'34" N 78°35'22" W	P	0.047	0.234	0.73
N. Myrtle Beach 1	33°49'28" N 78°38'55" W	HV	0.16	0.283	0.146
N. Myrtle Beach 2	33°48'50" N 78°40'49" W	HV	0.148	0.261	0.126
Myrtle Beach 1	33°42'11" N 78°51'47" W	HV	0.158	0.362	0.106
Myrtle Beach 2	33°40'01" N 78°54'21" W	HV	0.174	0.331	0.12
Garden City Beach	33°34'13" N 79°00'11" W	HV	0.136	0.318	0.233
Pawley's Island 1	33°26'19" N 79°06'53" W	MI	0.089	0.251	0.25
Pawley's Island 2	33°25'49" N 79°07'12" W	MI	0.083	0.233	0.228
Debidue Island 1	33°21'15" N 79°09'08" W	P	0.071	0.436	0.62
Debidue Island 2	33°20'09" N 79°09'28" W	P	0.089	0.384	0.71
Isle of Palm 2- 1	32°47'49" N 79°45'09" W	HI	0.16	0.234	0.246
Isle of Palm 2-2	32°47'24" N 79°46'19" W	HI	0.11	0.211	0.193
Isle of Palm 1-1	32°46'29" N 79°48'28" W	MI	0.094	0.208	0.392
Isle of Palm 1-2	32°46'33" N 79°48'39" W	MI	0.11	0.227	0.426
Sullivan's Island 1	32°45'43" N 79°49'32" W	HI	0.147	0.294	0.22
Sullivan's Island 2	32°45'23" N 79°50'24" W	HI	0.131	0.269	0.186
Folly Beach	32°39'33" N 79°55'36" W	MI	0.096	0.304	0.26
Burkes Beach	32°11'51" N 80°41'28" W	HI	0.097	0.329	0.206

## APPENDIX C:

### SUPPLEMENTAL TABLES AND FIGURES FOR CHAPTER 7

#### C.1 Tables

Table C.1. Study sites, impact level determined using the UI values, sample sizes for gut content analysis (n in 2016 and n in 2017) and sample size for stable isotope analysis (n for SIA).

Site	Impact level	n in 2016	n in 2017	n for SIA
South Island 1	P	9	-	-
South Island 2	P	7	-	-
North Island 1	P	10	-	-
North Island 2	P	10	-	-
Waties Island 1	P	-	12	11
Waties Island 2	P	-	12	12
Waties Island 3	P	-	11	11
Debordie Island 1	P	9	10	9
Debordie Island 2	P	-	11	11
Pawley's Island 1	MI	8	9	9
Pawley's Island 2	MI	7	8	8
Folly Beach	MI	8	6	6
Isle of Palm 1-1	MI	9	10	10
Isle of Palm 1-2	MI	-	12	12
Edisto Beach	MI	9	-	-
Surfside Beach1	HI	7	-	-
Surfside Beach 2	HI	9	-	-
Sullivan's Island1	HI	8	7	7
Sullivan's Island 2	HI	-	8	8
Isle of Palm 2-1	HI	7	8	8
Isle of Palm 2-2	HI	-	5	5
Burkes Beach	HI	8	9	9
Myrtle Beach 1	HV	4	5	5
Myrtle Beach 2	HV	6	4	4
N. Myrtle Beach 1	HV	6	5	5
N. Myrtle Beach 2	HV	6	4	4
Garden City Beach	HV	7	6	6



Table C.2. Results of post-hoc pairwise comparison following permutational multivariate analysis of variance (PERMANOVA) testing the variation in contribution of prey items to the ghost crab diet among disturbance levels (P= pristine sites, MI= moderately impacted site, HI = highly impacted sites by people, HV = highly impacted sites by people and vehicles).

<b>Comparison</b>	<b><i>F</i> - values</b>	<b><i>P</i> - values</b>
P - MI	1.88	0.159
P – HI	4.139	0.015
P – HV	7.837	0.013
MI – HI	0.512	0.702
MI - HV	1.565	0.223
HI - HV	0.528	0.732

Table C.3. Results of post-hoc pairwise comparison following permutational multivariate analysis of variance (PERMANOVA) testing the relationship between the availability and the contribution of prey items to the diet of ghost crabs within each disturbance levels (P= pristine sites, MI= moderately impacted site, HI = highly impacted sites by people, HV = highly impacted sites by people and vehicles).

<b>Prey item (disturbance level)</b>	<b><i>F</i>- value</b>	<b><i>P</i> - value</b>	<b>Prey item (disturbance level)</b>	<b><i>F</i>- value</b>	<b><i>P</i> - value</b>
Coquina clam (P)	314.3	0.009	Coquina clam (HI)	13.89	0.009
Mole crab (P)	34.1	0.007	Mole crab (HI)	8.431	0.007
Algae (P)	15.39	0.008	Algae (HI)	12.41	0.008
Cannibalism (P)	17.76	0.008	Cannibalism (HI)	11.03	0.008
Misc. animal (P)	27.43	0.007	Misc. animal (HI)	4.188	0.023
Coquina clam (MI)	9.803	0.009	Coquina clam (HV)	3.768	0.051
Mole crab (MI)	12.6	0.007	Mole crab (HV)	6.496	0.048
Algae (MI)	78.64	0.008	Algae (HV)	10.63	0.008
Cannibalism (MI)	9.732	0.008	Cannibalism (HV)	3.075	0.047
Misc. animal (MI)	8.137	0.007	Misc. animal (HV)	2.142	0.125

## C.2 Figures

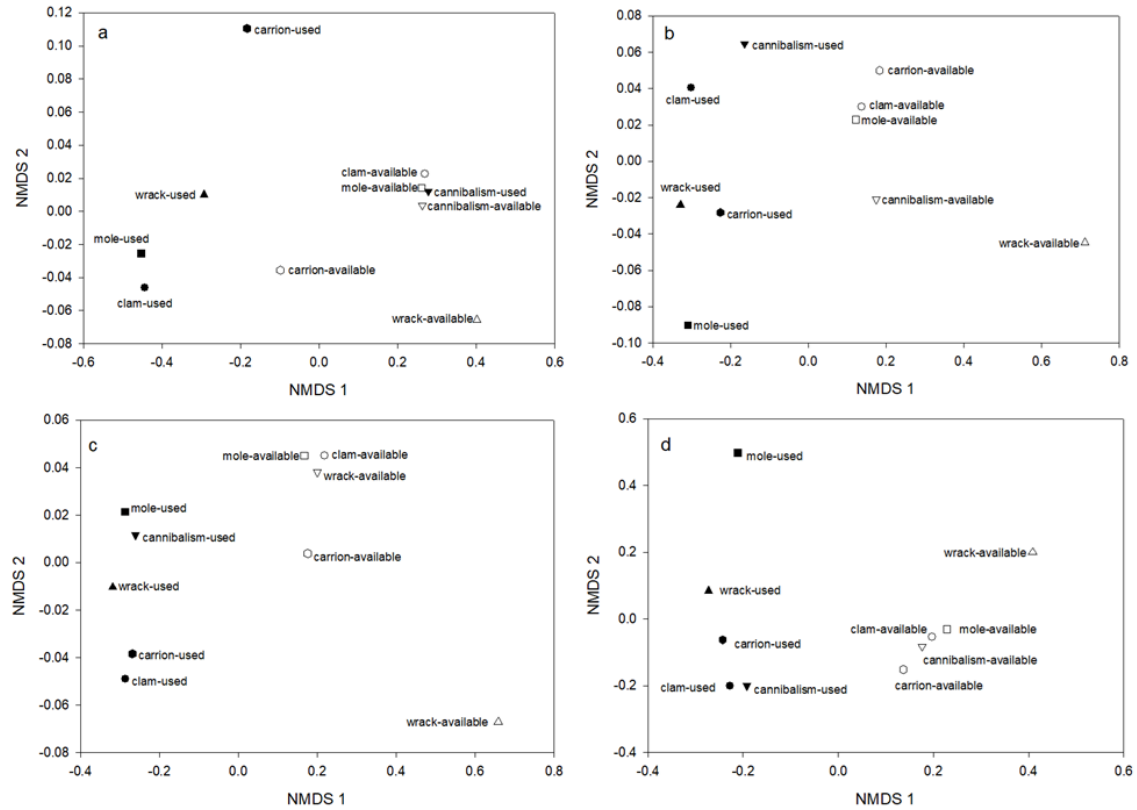


Figure C.1. MDS plots for the availability and the contribution of the prey items to the diet of ghost crabs (a=pristine sites, b=moderately impacted sites, c= highly impacted sites by people, d= highly impacted sites by people and vehicles).

## APPENDIX D:

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